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BACTERIAL SPORES

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(*Received* April 27, 1931.)

(With Three Text-figures.)

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I. INTRODUCTION.

APART from its considerable practical importance, the process of spore formation in bacteria is of great interest in relation to the cytology and life history of these micro-organisms. The resistance of spores to conditions unfavourable for the majority of organisms has played an important rôle in the history of bacteriology. The process is also of interest in affording an example of the dangers of pushing teleological considerations too far.

The word spore (σπόρος, a seed) made its first appearance in botany and was used to define the small bodies functionally corresponding to the seed in what were known as the agamous plants, *i.e.* plants with no embryo. For an historical account of the use of this word see de Bary (1887, p. 131). The earlier developments of bacteriology being in the hands of the botanists it is not difficult to understand how this term became applied to the frequently refractile oval or spherical bodies formed in the cell of numerous species of rod-like bacteria (bacilli) and which are different in structure and properties from the parent cell. The word as used for bacteria appears first in Cohn (1876). These intracellular spores are sometimes called endospores. The subject of this review is concerned mainly with these forms. Certain other forms occurring in bacteria and which are sometimes known as spores will be dealt with in a later section. The formation of spores in moulds is of rather a different nature to that observed in bacteria, but a somewhat similar process takes place in the encystment of protozoa and in yeasts.

It must be impressed that the process of endospore formation is not of general occurrence in all types of bacteria (Schizomycetes). The vexed question of the classification of these organisms does not come within the scope of this review except in so far as endospores are characteristic of certain groups. The classification and nomenclature of the organisms described is essentially the same as that adopted by the first American Committee on Bacterial Nomenclature (1920). An examination of this classification shows that endospore formation occurs characteristically only in the family Bacillaceae with the two genera *Bacillus* and *Clostridium*. The process has been described in the family Spirillaceae, but it is not of general occurrence in this group. It is possible that endospore formation occurs in other types of bacteria, but these forms are generally so small that no definite alteration in structure can be observed. The balance of evidence, however, is against this possibility.

The form, size and situation of the spore within the bacterial cell is a fairly constant property of various species, and these characteristics are used in the classification of the organisms. The shape is generally spherical or oval, but other forms may occur. The diameter of the ripe spore is sometimes greater than that of the parent cell, and if the spore is situated equatorially the form is known as clostridium, and if terminally, plectridium. In the majority of spore-forming bacteria, however, the spore is of lesser diameter than the parent cell although the position may vary. The size varies according to the species. Further details of this nature will be found in manuals of Descriptive Bacteriology such as that of Bergey (1923). It is to be noted that, while the majority of spore-forming bacteria are motile, the spores are non-motile. This is, no doubt, due to the fact that the spores do not possess flagellae or cilia.

The habitat of most, if not all, of the spore-forming bacteria is the soil. The subject of the habitat of the organism and its capacity of forming spores will be dealt with in a later section. It is interesting to note, however, that the spore-forming bacteria constitute only a small proportion of the total number of organisms present in the soil.

The first observation on spore formation in what would now be considered to be a bacterium was by Perty (1852). He noted the formation of oval bodies in the cellular material of a large water bacillus. He evidently recognised the nature of the change as he termed the organism *Sporonema gracile*. Pasteur (1870) noted the formation of highly refractile bodies in the micro-organism that was thought responsible for pébrine (lethargy in silkworms). There is no evidence that Pasteur realised the significance of these forms. Cohn (1872) also observed these refractile bodies, but not until 1876 did he publish the results of his studies on this process in a bacillus obtained from hay infusions. He followed the formation of the spore and showed that it was capable of germination. The resistance to heat was studied and the conclusion was drawn that the spore was the persisting form of the organism. The process was studied later in greater detail in *B. subtilis* by Brefeld (1881), in *Cl. butyricum* by Prazmowski (1880) and in *B. megatherium* by de Bary (1884). Other observations in relation to special features will be mentioned later.

It is a point of great interest that spore formation was noted in *B. anthracis* by

R. Koch (1876). Koch, working more or less under the guidance of Cohn, made a very careful study of the life history of this organism. Topley and Wilson (1929) point out that the existence of an endospore and its significance in reproduction having been demonstrated in the case of this bacillus, which was one of the first to be identified as the cause of an important infective disease, led many workers to approach the study of a new bacterial species with a bias towards identifying any morphologically differentiated element in the cell as a spore. For this reason much of the earlier observations are full of mistaken allusions to spores or sporogenic granules. It is evident that the test of what is a spore must remain its capacity of germination.

II. THE CYTOLOGICAL CHANGES INVOLVED.

It is evident that in such a process as spore formation considerable changes must take place in the cell. It is interesting to note that the evidence as to the existence of nuclear structure in bacteria has been almost exclusively based on the changes occurring in cell structure during sporulation. Before this evidence is examined a description of the main characteristics of the process is necessary. The earlier workers cited in the introductory remarks were not concerned with the minuter details of cellular structure and the process was usually followed in living preparations. The following description is taken from de Bary (1884), and as an account of the essential details of the process it could not be bettered. The bacillus studied was *B. megatherium* in a hanging drop preparation. de Bary wrote: "The commencement of the formation of a spore in a cell is indicated by the appearance of a small roundish highly refringent body in the protoplasm, usually close to the surface of one extremity of the cell...the body then grows perceptibly larger while the protoplasm round it diminishes. In the course of a few hours it grows into a longish cylindrical object, which is shown by its subsequent behaviour to be a spore. The spore is slightly shorter than the cell which produces it, but its breadth is only from a third to one-half of it."

The cytology of the bacteria has never been studied extensively, and the relatively gross morphological changes that are noted, such as in the above account of the process, have been taken to indicate the existence of nuclear elements. It is not intended to give an exhaustive account of this subject here. The earlier literature is reviewed by Migula (1904) and Dobell (1911) and the more recent developments are given by St John-Brooks (1930). The matter is still extremely controversial. This position is not difficult to understand, as apart from the small size of the organisms under observation the evidence has been derived from preparations which have been fixed and stained by so many different methods that no certainty can be attached to the significance of the structures observed. It is possible that the use of ultra-violet illumination for the microphotographic study of bacteria in the living state will throw light on their structure (see Barnard, 1930).

The idea of a diffuse nuclear structure in bacteria has been developed mainly by Schaudinn (1902, 1903), Guilliermond (1906, 1908) and Dobell (1908, 1909 and 1911). It seems to the writer that the evidence of these workers is undoubted, at any rate for certain organisms. Dobell (1911) has written a critique of the staining methods used by previous workers. He points out that the cytological significance of a given structure must be judged from the changes which it undergoes rather than from its affinity for a particular dye. Hence an account of some of the changes observed during spore formation is of interest. The observations summarised below are typical of a much larger amount of work to which the writers cited above give complete references.

Ernst (1888, 1889) noted in *B. xerosis* the formation of granules which stained with methylene blue and Bismarck brown. He stated that these granules fused to form the spores. For this reason he called them sporogenic granules. It was shown, however, by later work that they did not play any rôle in this process. These Babes-Ernst granules or metachromatic granules are probably of the nature of a nitrogen reserve material. There are excellent reviews on the subject of these granules by Guilliermond (1906) and Zettnow (1918) who gives some excellent figures of the structure of various bacteria. It appears that these granules make their appearance in the cells of fungi, algae, protozoa, etc., and it can be definitely said that they play no rôle in spore formation. Schaudinn (1902) followed the life history of *B. bütschlii*. He concluded that in this bacillus the nucleus is in the form of scattered granules of a chromatic substance (chromidia) throughout the greater part of the life cycle. During spore formation the granules arrange themselves in a spiral and finally become aggregated into dense masses in the fully formed spore. A process interpreted as a modified sexual process was observed. This subject will be dealt with in a later section. In 1903 analogous conditions were found in a marine organism *B. sporonema*. Wahrlich (1891) was of the opinion that in *B. subtilis* and *B. megatherium* the cells contained chromatin and that the chromatin granules fused to form spores. Preisz (1904) made an extensive study of *B. anthracis* and maintained that nuclei hitherto described were really only portions of the cytoplasm that had become more deeply dyed. He stated that real nuclei were present in the form of minute spherical corpuscles, one or more in each cell; these undergo division and a nucleus enters into each spore. He noted similar nuclei in *B. cohaerens*, *B. tetani* and *B. asterosporus*. Guilliermond (1908) believed that in *B. radicosus* and *B. mycoides* the nucleus was in the form of granules of chromatin (chromidia) scattered through the cytoplasm, which were distinct from the metachromatic granules. These granules became massed to form the spores. Dobell (1908) found in a large disporic bacillus, *B. flexilis*, from the gut of frogs and toads, a life history similar to that of *B. bütschlii*. In another organism the nucleus was in the form of spiral or zig-zag filaments. In *Spirillum monospora* the nucleus was of chromidial form. The spores are formed by the massing of chromidia. The process was described in greater detail in 1909 for *B. spirogyra* and for another organism *B. lunula*. In 1911 an account was published of the life history of a large number of organisms obtained from the gut content of various amphibia and reptiles. It was found that the nuclear pattern differs considerably. Dobell concludes that the bacteria are nucleate cells. The form of the nucleus, however, is variable not only in different bacteria but at different periods in the life cycle of the same species. It is evident with considerations of this sort that the bacteria are only a provisional grouping and are probably heterogenous. Both Guilliermond and Dobell point this out and suggest that a study of the cytology will help in the classification of these micro-organisms. This aspect of spore formation is of great biological interest, and may help us to understand the significance of the process. But it must be remembered that the majority of bacteria do not form spores, and it is doubtful whether the cytology of this majority resembles that of the spore formers.

In distinction to the above account of a diffuse nuclear structure some observers have described a well-defined nucleus. Meyer (1897, 1899 and 1908) is the chief exponent of this view. He noted in numerous species of bacteria but especially in *B. asterosporus* the existence of one to six granules in each cell. These are taken to be nuclei. At the moment of formation of the spore a nucleus is present. The spore is formed as a vacuole in which the easily colourable cytoplasm condenses and into which the nucleus penetrates. Similar accounts have been given by other workers (Nakanishi, 1901, Ellis, 1922).

III. FACTORS INFLUENCING SPORULATION.

The view has been commonly adopted in bacteriology that the formation of spores corresponds with the moment when the culture medium has been exhausted of its supply of nutrient material, when there is an accumulation of the products of growth, or when the development of an unfavourable reaction renders the medium unable to support further growth. Though most spores are resistant to adverse conditions this teleological explanation does not altogether explain the process. Buchner (1880, 1890) believed that spore formation was caused by the utilisation of all the available food material. This view was also taken by Schreiber (1896), Matzuschita (1902) and Kruse (1910). This view appears to be the one most commonly adopted in text-books of bacteriology. Buchner from a consideration of the study of *B. anthracis* explained the process as being due to a local exhaustion of the food supply; although as Stephenson (1930) points out none of his experiments were sufficiently rigorous to exclude the possibility of other factors such as change of reaction, unsuitable salt balance, etc., from being contributory or even primary causes. The conclusions of Buchner were contested by Lehmann (1887) and Osborn (1890), both working with the same organism. These workers showed that utilisation of food material did not necessarily bring about spore formation. Turro (1891) explained the process as being due to the formation of metabolic products. The supposition of Buchner has been the basis of such laborious experiments as those of Schreiber (1896), Matzuschita (1902), Selter (1904), and Holzmüller (1909). It is interesting to note that Demnitz and Weyrauch (1925) observed no effect of filtrates of old anthrax cultures on freshly growing cultures of this organism.

The effect of oxygen tension on the process in *B. anthracis* was studied by Klett (1900) who found that spore formation occurred on all ordinary media under anaerobic conditions. Jacobitz (1901) was unable to repeat this result and thought that Klett's observations were due to the fact that some oxygen had been left in the medium. Weil (1899) observed spore formation in *B. anthracis* cultures in an atmosphere of hydrogen when the organisms were cultivated on potato and other vegetable media and in coagulated sheep blood serum plus 25 per cent. glucose, but not on other media. Holzmüller (1909) found that oxygen appears to act as a direct stimulus to spore formation in the *mycoides* group. The effect of oxygen tension on the sporulation of bacteria and the germination of spores has been the subject of elaborate researches. As is well known the oxygen requirements of different organisms vary. The literature on the subject of oxygen tension and growth and spore formation in bacteria is given by Kruse (1910, p. 96) and by Waksman (1927, p. 162). It seems to the writer that no definite conclusions can be drawn as to the effect of oxygen tension on either sporulation or on germination. It appears, however, that any effect is correlated with the oxygen requirements in so far as they influence the growth of the bacteria.

It has already been noted that spore formation is usually taken to occur at the moment when the medium has for some reason become unsuitable for further growth of the organism. Prazmowski, however, noted that in *Cl. butyricum* some of the cells may be in active process of vegetative multiplication while other cells are forming and maturing their spores. A similar observation has been made under dark ground illumination with preparations of *B. subtilis* and *Cl. sporogenes* by Miss M. Stephenson and the writer. It is clear, therefore, that the process does not occur in a consecutive manner.

The effect of the addition of various compounds to the media and their influence on sporulation is of interest. A. Koch (1888) observed that hanging drop preparations of *B. inflatus* form spores in 1–2 per cent. solution of nutrient broth, but that the spores are not produced when glucose is added to the medium. Fitzgerald (1911) found that in bacilli belonging to the *aerogenes-capsulatus* group (*Cl. welchii*) raffinose and mannitol appeared to favour sporulation. Other sugars, such as *d* glucose, sucrose, maltose and lactose had no effect. From this group of sugars acid was produced as a result of the bacterial metabolism, and this appeared to have an inhibitory effect on spore formation. An initial alkaline reaction appeared to favour spore formation. The process occurred normally in sugar-free media, and it is interesting to note that different strains showed different capacities to sporulate. Torrey, Kahn and Salinger (1930) investigated the effect of hydrogen-ion concentration on spore formation in *B. welchii*. A sugar-free, well-buffered medium was studied, and it was found that spore formation takes place in the region from *p*H 6·8 to the alkaline limit for its growth. Spore formation was entirely checked during the first week below *p*H 6·6. Contrary to the results of Fitzgerald, raffinose and mannitol did not favour spore production.

Cook (1931) found that in *B. subtilis* spore formation took place under conditions of good growth of the organism and when an unfavourable environment did not afterwards develop. Thus in fluid tryptic broth medium, although the initial growth was good and spore formation was observed after 2 to 3 days, autolysis set in after about 6 days. This autolysis was no doubt caused by the enhancement of the normal proteolytic activity of the organism by its being kept in this medium. A similar result was obtained in peptone water. In an inorganic medium to which 1 per cent. glucose had been added good growth was observed in the earlier stages and some spores were observed, but after some time the medium became acid and the organisms were lysed. In inorganic media to which 1 per cent. glycerol or sodium lactate had been added good growth was noted, and at the end of 35 days nearly all the forms observed were spores. The experiments were carried out in liquid media and in media to which 2 per cent. agar had been added. These were made up in the form of agar slopes. Far better spore formation was observed on these solid media. Very large spores were noted in the lactate medium to which peptone was added. As the addition of peptone usually increases autolysis the formation of these spores might be explained by their development in the earlier stages of growth and their resistance to the proteolytic enzymes. The effect of various different hydrogen-ion concentrations was also studied, but it would seem that spore formation only occurs under the best conditions of growth and where autolysis does not set in. In this connection the work of Daranyi (1927) is of the greatest interest. Daranyi worked mainly with *B. anthracis* but also with *B. subtilis* and *B. anthracoides*. He concluded that the ability of the organisms to sporulate depends in general upon the same optimum conditions which lead to good vegetative development of the bacteria. Further experiments of this worker will be dealt with later on.

Migula (1904) investigated the effect of temperature on the process and found that spore formation is less rapid and complete on either side of the optimum temperature for growth. He found also that spore formation seems to depend upon the density of the population in the culture. He stated that spore formation can be indefinitely postponed if the culture is transferred to a new medium at a period preceding the appearance of spores. Migula's view was that spore formation begins after a definite number of cell divisions have taken place. The process appears at a definite concentration of cells regardless of the initial amount of inoculum and begins when the medium is no longer suitable for vegetative growth, that is when growth has ceased. Migula found also that spore formation could not be delayed by the addition of a concentrated form of nutrient such as dry peptone or meat extract, but on the addition of distilled water to the medium spore formation was postponed. His conclusion was that spore formation is not due to an exhaustion of the medium, but that it depends entirely upon the concentration of the cells and is probably the result of an accumulation of the products of metabolism of the bacteria.

The careful work of Henrici (1924, 1928) is very illuminating on the factors influencing spore formation. The experiments were carried out on *B. megatherium*. The results obtained are shown in Fig. 1. It will be seen that spore formation commenced practically at the time when the logarithmic growth phase passed over to the resting phase. After spore formation commenced it proceeded at a practically constant rate for some time. The rate of spore formation began to slow down after some hours, the rate continually decreasing during the period of observation. It is evident therefore that all the cells do not develop into spores. As spore formation appears to be a characteristic of the resting phase the rate of spore formation should be influenced by those factors which also tend to influence the rate of growth and the form of the growth curve. Experiments were carried out on *B. cohaerens* to test this point. A culture with few vegetative forms was inoculated on to four sets of agar slopes; two of normal composition and two containing one-fourth of the quantity of peptone and meat extract. One set of each kind was inoculated with a very heavy suspension and the other with the same suspension diluted fifty times. Samples were removed at intervals and the percentage of free spores determined. It was found that spore formation proceeded more rapidly in the media of lower nutritive value regardless of the size of seeding and that it proceeded more rapidly in the heavily seeded cultures than in the lightly seeded ones, though the nutrient material exerted a greater influence than the amount of seeding. In the heavily seeded dilute medium spores never entirely disappeared, new spores starting to form before all those introduced had germinated. It would therefore appear that the rate of spore formation is determined not by the concentration of cells alone, but rather by the density of the population in relation to the concentration of foodstuff in the medium. It appears earlier in the heavily seeded cultures and in the more dilute media because these cultures do not have so long a period of vegetative reproduction; that is because the resting phase of the culture appears earlier. Williams (1930, 1931) has found that although the total amount of growth of *B. subtilis* is greater in 5 per cent. peptone water than in 1 per cent., the ratio of spores to vegetative forms at the end of 10 days is very much lower in the more concentrated medium. It is not intended to deal with the significance of the above observations at this stage, as they will be treated in conjunction with other points in a later section.

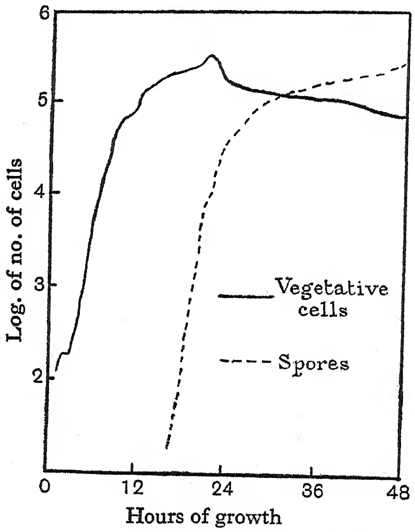

Fig. 1. Growth and spore formation in *B. megatherium* (Henrici, 1924).

The effect of the addition of various salts has been investigated by a number of workers (Matzuschita, 1902; Fitzgerald, 1911; Daranyi, 1927; Cook, 1931), but it does not appear that salts exert a direct influence on spore formation. Matzuschita has compared the concentrations of sodium chloride which limit the growth and spore formation of various bacteria, but it is doubtful whether the slight differences are really significant. Fitzgerald found that the addition of this salt in concentrations ranging from 0·5 to 5 per cent. did not affect spore formation in the *aerogenes-capsulatus* group. Behring (1899) noted that spore formation appeared to be induced in cultures of *B. anthracis* by the addition of a solution of calcium chloride. Daranyi has confirmed this with the same organism and with other bacteria. He also finds that exposure of the bacteria to alcohol vapour induces sporulation. Daranyi is of the opinion that spore formation is brought about primarily by colloidal reactions, the most important of these being a diminution in the water content of the organisms resulting in a shrinkage of the colloids. Calcium chloride and alcohol vapour would bring about this condition by virtue of their dehydrative action. Additional evidence is also found in the fact that greater amounts of spores are formed at the upper end of agar slopes, where the medium would be expected to be drier. This is an observation which was also made by Kruse (1910). It was also found that a greater percentage of spores were formed when the organisms were grown on plates which have a greater surface than slopes, and thus facilitate drying. Daranyi claimed to have brought about sporulation in young cultures of *B. anthracis* by dehydration with calcium chloride and with alcohol vapour. He noted, however, striking differences in the capacity to sporulate of the different strains he investigated. Michailowsky (1926) studied the effect of a large number of organic solvents such as alcohols, aldehydes, essential oils, etc., on *B. anthracis*, *B. mesentericus*, *B. subtilis* and *B. megatherium*. He found that of these various compounds the lipoid solvents (chloroform, bromoform, amyl alcohol, petrol ether, toluene and benzol) all appeared to exert a favourable action on spore formation. Added to 36-hour cultures in some cases complete spore formation occurred. The significance of this curious observation will be discussed later.

It will be seen from the work described in this section that it is impossible to state definitely that spore formation is brought about by reason of any one physical or chemical effect.

IV. THE PROPERTIES OF SPORES: THEIR COMPOSITION AND METABOLISM.

Perhaps the most striking property of spores is their resistance to the temperature of boiling water. The celebrated discussion on Spontaneous Generation perhaps owes its long duration to the existence of such spores. An excellent account of this subject is given by Bulloch (1930). That forms of life exist which are capable of germination after having been boiled was first shown by Spallanzani (1777). The matter was carefully investigated by Pasteur (1861) who found that the spores of moulds were capable of germination after being heated *in vacuo* or in dry air for an hour. At 100° in moist air, however, this capacity was lost. Roberts (1874) showed definitely that organisms existed in vegetable matter which could withstand this temperature. His conclusion was that they were bacteria or their germs. Cohn (1876) confirmed these observations. The subject was taken up by Tyndall (1877) and put to a thorough test. He found that even after boiling for 5½ hours certain vegetable infusions showed signs of growth on incubation. This power of resistance to high temperatures varies with the conditions to which the spores are exposed and depends on the species. Thus anthrax spores are killed by 10 minutes' boiling, while the spores of *B. subtilis* are still capable of germination after as long as 6 hours' boiling. That this power of resistance depends in part on the reaction of the medium was shown by Pasteur (1860). He found that whereas acid infusions have their germinal life destroyed at 100°, a temperature of over 100° was required to sterilise infusions with an alkaline reaction. A further account of this subject was published in 1875. Pasteur's conclusion was: "...que l'acidité permet et que l'alcalinité empêche la pénétration de l'humidité dans l'intérieur des cellules germes, associées aux infusions, de telle sorte que chauffer les enveloppes de ces cellules ou les parois de leurs kystes dans un milieu alcalin, c'est chauffer les germes à l'état sec; les chauffer dans un milieu acide, c'est les chauffer à l'état humide, et l'on sait qu'il y a sous ce rapport une grande inégalité dans la résistance à la température." The resistance to heat of various bacteria and spores has been the subject of numerous investigations. The importance of a knowledge of these conditions in the preparation of sterile culture media and apparatus needs no stressing. Detailed accounts of the effect of heat on micro-organisms will be found in Lafar (1907) and Topley and Wilson (1929).

It has been found that the spores of the same organism differ in their thermal resistance. Magoon (1926, 1) found that this property was influenced by the age of the spores, the temperature and humidity of the environment. It was found that the highest resistance to heat develops under conditions of moderate temperatures and humidity and is reached by the time the spores are 60 days old. Spores of different species, however, differ in this respect. Magoon (1926, 2) found also that the spores derived from the resistant survivors in thermal death time tests possessed a greater resistance to heat than the original spores, and by a process of selection a strain could be obtained which resisted a temperature of 100° for twenty-five times longer than the original spores. Williams (1929) found that *B. subtilis* formed spores of enhanced resistance in peptone water to which had been added salts of magnesium, phosphates or certain carbohydrates. Cultivation in iso-electric gelatin and in casein digest gave very resistant spores. Higher temperatures of incubation had the same effect. No correlation was found between the extent of growth or of sporulation and the resistance of the spores.

The effect of light has been studied by Ward (1895), Schreiber (1896) and Holzmüller (1909). It would seem, however, that the effect is much the same as that on the vegetative forms, allowing for a slightly greater resistance on the part of the spores. This subject is dealt with in the manuals previously cited.

An excellent account of the effect of disinfectants on bacterial spores and on bacteria in general is given by Chick (1930). In general the spores are more resistant to the action of disinfectants than the vegetative forms of the organisms.

Spores remain alive for a considerable time. The earlier workers noted that spores were capable of germination after several years. Székely (1903) found that anthrax spores were alive after 18 years. It is to be noted, however, that a culture of the bacillus causing malignant oedema was also virulent at the end of 18½ years. Spores of most organisms remain alive even under conditions when the cultures become dry. In fact dry spores if kept in an unchanging environment will remain viable for long periods. It is to be noted that the non-spore-forming organisms remain alive for considerable periods if kept in a suitable environment (such as a sealed agar stab culture). But spores are by no means deathless. Swann (1924) found that about 5 per cent. of young anthrax spores were dead after 2–3 days, and about 55 per cent. of old dried spores were dead after 1 year.

The composition and structure of spores. The composition of spores has not been studied in great detail. The refractile appearance on formation has given the impression that these bodies contain a large percentage of lipoid substances. Ward (1895) noted that the oil-like globules which appear in *B. ramosus* and fuse to form the spores are not oil droplets because they stain with methylene blue and other dyes which have chromatin affinities. It has also been noted that the globules do not stain with Sudan III or other dyes with lipoid affinities.

It is generally considered that the spores have a very low water content. In mould spores this is undoubtedly the case. Cramer (1891) found that the dry weight of spores of *Penicillium crustaceum* was 61·13 per cent. and that of the mycelium 12·36 per cent. Sumi (1928) found a water content of 17·43 per cent. for the spores of *Aspergillus oryzae*. The composition of these spores is given by this author, and numerous analyses of mould spores are given in Lafar (1907). The only bacterial spores that seem to have been investigated are those of *B. anthracis*. Dyrmont (1886) found that the percentage dry weight of spores was only 14·6 per cent. as compared with 20 per cent. for the vegetative form. Kruse (1910) pointed out that this result might be due to the fact that the spores lie embedded in a gelatinous mass formed from the cell during sporulation. It is evident that the substance of the spore is in a more concentrated form than that of the vegetative form. Almquist (1898) found the specific gravity of *B. subtilis* spores to be 1·35–1·40. Stigell (1908) found the specific gravity of the vegetative form to be 1·12. It is evident from these figures that lipoids do not form a large part of the spore substance, a conclusion which is confirmed by the analyses of Dyrmont. This worker found that the percentage of substances extracted by lipoid solvents was much the same for the spore as for the vegetative form (8·7 and 7·1 per cent. respectively). The nitrogen content expressed in terms of dry weight of organisms was 10 per cent. for the spores and 6·3 per cent. for the vegetative form. In moulds the difference between these figures is not so marked and it is possible that in some bacteria at least the nitrogenous constituents of the cytoplasm are concentrated in the spore.

The structure of spores. Owing to the small size of the spores it is difficult to see the structural elements. The spore appears to be homogeneous. There is no doubt that the spore possesses a definite membrane, but the structure (*i.e.* whether it consists of two layers) and the composition are largely conjectural. According to de Bary the spore of *B. megatherium* is closely surrounded by a thin but firm and often brittle membrane; outside the cell wall is often seen a pale slightly refringent envelope with lightly marked contour and apparently gelatinous consistence. This forms a delicate covering to the spore, and appears sometimes to be prolonged at each extremity of the spore into a small tail-like appendage.

In regard to the formation of an impermeable membrane round the spore during sporulation it is interesting to note that although before the formation of the spore the cell readily takes up the ordinary bacterial dyes, this property is gradually lost and later on only the outer surface of the membrane is tinged with the dyes. The resistance of spores to ordinary stains forms one of the means of differentiation of these forms. Details of the methods commonly used are given in all practical text-books of bacteriology.

In *Cl. butyricum* and *Spirillum amyliferum* granules are seen which become blue with iodine. In those forms which become more highly refractile in the stages preceding spore formation the protoplasm assumes a blue or violet colour with a solution of iodine, either throughout or with the exception of certain transverse zones which do not turn blue. In both cases the substance which has become blue is spread uniformly throughout the protoplasm without forming bodies of a definite shape. This amyloid substance disappears on the formation of spores.

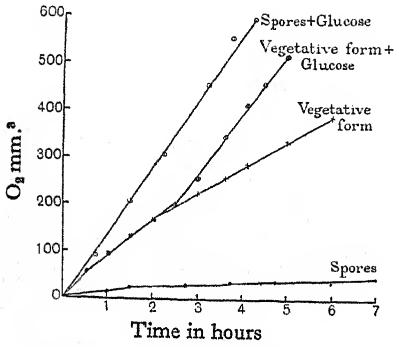

Fig. 2.

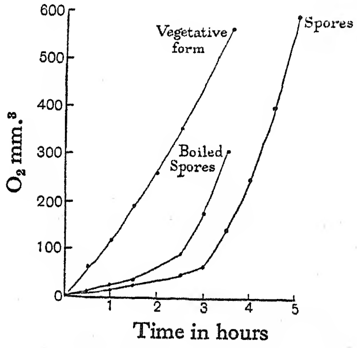

Fig. 3.

Fig. 2. The respiration (measured as oxygen intake) of the spores and vegetative form of *B. subtilis* with and without *M*/300 glucose at 40° C. and *p*H 7·2. Nitrogen content of spores in each experiment 0·59 mg., of vegetative form 0·55 mg. (Cook, 1931).

Fig. 3. The oxygen intake of the vegetative form of boiled spores and of normal washed spores of *B. subtilis* in 1 c.c. of tryptic broth medium at 40° C. and *p*H 7·2 (Cook, 1931).

The metabolism of spores. This subject has not been investigated extensively. The general impression has been that these forms are a resting stage in the life cycle of bacteria and are therefore not very active metabolically. Kopeloff and Kopeloff (1919) found that spores of *Aspergillus* produced invertase. Effront (1917) found that bacterial spores, attenuated either by an antiseptic or by heat, are the more productive of proteolytic enzymes the more difficult is their germination. Ruehle (1923) found that the spores of certain aerobic bacteria showed a marked proteolysis of gelatine and a marked catalase reaction, but the oxidase, reductase (methylene blue reduction) and lipase reactions, and also the digestion of casein, were negative.

Cook (1931) found that spores of *B. subtilis* showed a marked proteolysis of gelatine. They do not reduce methylene blue except in the presence of glucose. However, the vegetative form does not reduce methylene blue with any potential hydrogen donator, glucose included. The respiration of the spores as compared with the vegetative forms is shown in Fig. 2. The material after having been taken off the agar was always well washed with Ringer's solution, so the residual respiration would be expected to be small. On the addition of glucose or sodium lactate, however, the respiration increases. Fig. 3 shows the respiration in tryptic broth. In this case growth sets in, and it will be seen that the vegetative form shows no lag. It is interesting to note that the lag is less with the spores which have been boiled previous to the experiment than with the spores which have been simply washed. Experimental details are given in the original paper.

V. GERMINATION.

The method of germination of spores is not such a vexed question as their formation. In general there is a considerable degree of constancy in the process for spores of a given species. This fact may be used in the differentiation of bacteria and is confirmatory of the nature of the spores. The spores germinate when placed in a suitable culture medium. The process may be of three main types. The first stage is much the same in all types. The spore becomes distended and the refractile appearance disappears. In the first type, of which *B. anthracis* may be taken as a typical example, the process is very simple. The spore gradually acquires the normal dimensions of the vegetative form which then divides by fission. In the second type, which is observed in *Cl. butyricum*, the spore capsule opens at one of its poles so that the direction taken by the growing bacillus is in a line with its length. The expulsion is effected by the spore membrane, the tension of which is gradually increased to such an extent through the expansion of the spore content that it finally forces out the grown bacillus. The capsule shrinks to its original size and finally disappears in the medium. In a species described by Klein (1889), *B. sessilis*, the mature bacillus remains embedded in the spore membrane. This, however, does not affect the growth of the organism. The third type of germination is shown by *B. subtilis* and *B. megatherium*. The spore membrane does not burst at the poles, but at a line coinciding with the equator of the spore. This line does not extend right around the spore, so that the two halves of the membrane still remain attached together at one point. The bacillus is then pushed out of the spore membrane. According to the careful observations of Brefeld (1881) in *B. subtilis*, the outer wall of the spore, which is comparatively thick and continues to be highly refractile, splits across the middle in germination after its first distension into two portions which, however, remain firmly united on one side. The protoplasm elongates in the direction of the longer axis of the spore and of the longer axis of the mother cell which coincides with it, and at the same time usually bends through about 90°, and one extremity is thus thrust out of the opening in the outer wall of the spore while the other extremity remains in the spore. The wall of the spore which has opened on one side is evidently very elastic; it manifests so considerable a resistance to the elongating rod as it bends, that the rod with both its extremities fixed in the spore becomes curved before one extremity is set free. The resistance sometimes goes so far that both extremities remain fixed in the spore, and in that case the elongating bacillus assumes the form of a horse-shoe, the limbs of which may be of considerable length.

The conditions for the germination of spores necessarily depend on various factors and depend also on the species under investigation. On suitable nutrient material the optimum temperature for germination in *B. subtilis* is 35°–38° (Migula, 1904). Complete germination takes place in 5–7 hours. At 12° the process takes at least 2 days. Prazmowski found that the optimum for *Cl. butyricum* was 30°–35°. de Bary found that spores of *B. subtilis* germinated readily at 40°. *B. anthracis* will not germinate at room temperature, the minimum appearing to be in the region 35°–37°. *B. megatherium* was found to germinate at 20°–25° in 8–10 hours. Ward (1895) found that a water bacillus investigated by him, *B. ramosus*, readily germinated at 25°. Holzmüller (1909) found that the spores of the *mycoides* group germinated at temperatures between 8° and 53°, but he noted that at the extremes the process is imperfect, and that the optimum region was from 28° to 35°. It is probable that the optimum is the same as for the vegetative growth, and varies with the species according to the conditions pertaining in their normal habitats.

The importance of water for the process needs no stressing. As the process is usually followed in an aqueous medium, and the system rapidly attains equilibrium with the liquid phase, little is known about the conditions under which water enters the system. The influence of humidity on the germination of mould spores has been studied by Tomkins (1929). He has found that when moulds are germinating on a substratum in equilibrium with the atmosphere, the range of humidities over which germination is possible varies with the temperature. It would be of interest to study the relation to these conditions of an organism whose general habitat is not in an aqueous milieu. Such organisms would include those normally found in the soil.

The subject of the effect of the osmotic pressure of various salts and other compounds on the germination of spores is of some interest. Holzmüller found that the spores of the *B. mycoides* group would only germinate within a narrow range of osmotic pressures corresponding to the optimum for vegetative growth. Eijkman (1918) noted the effect of various substances on the germination of anthrax spores. The osmotic pressure of the culture to which the substances were added was measured as the lowering of freezing point. The spores were sown into a culture medium in which growth normally took place, and the concentration of salts and other compounds that prevented germination were noted. Some of the results obtained are shown in Table I.

In the first group were found various salts that inhibited the germination of spores at approximately the same osmotic pressure. Other substances mixed with NaCl give approximately the same result (group 2). The third group includes substances which do not inhibit in proportion to their osmotic pressure. Some of these prevent germination in small quantities. Others have no effect even in large amounts. NH_4Cl and Na_2HPO_4 inhibit by their toxic action. This can be overcome by using them in small amounts and combining them with NaCl. Other substances such as urea, methyl and ethyl alcohols and glycerol added in concentrations of 1–2 per cent. do not sensibly lower the limit of concentration of NaCl. They do not exert an osmotic pressure on the spores. These substances are incapable of plasmolysing the vegetative cells. The explanation is that the cell is permeable to these compounds which penetrate into the cell and do not influence the distribution of water. Eijkman's conclusion is that "the impression is received that the spores are not so isolated from their environment as perfectly as one admits in general."

Table I.

	Substance	Concentration (%)	Δ*
I.	NaCl	4·5	− 3·46
	KNO_3	9·1	− 3·24
	Glucose	23·4	− 3·56
II.	Mannitol	16	− 3·26
	+		
	NaCl	1·5	
III.	NH_4Cl	2·5	− 2·48
	NH_4Cl	1·3	− 3·70
	+		
	NaCl	2·5	
	Urea	7·1	− 2·82
	Urea	1	
	+		
	NaCl	4·5	

* These values appear to have been determined with the substances mentioned in solution in the culture media.

Fischoeder (1909) investigated the germination of anthrax spores. Old cultures were washed off nutrient agar and heated to 80° for 3 minutes to kill off vegetative forms. It was found that about half the spores germinated in physiological saline in 5 hours, in tap water the process was more rapid, while in broth about 98 per cent. of the spores had germinated in 10 minutes. Stephenson (1930) pointed out that from the method used differentiation between true germination and the passing from a heat stable to a heat labile condition could not be made.

Fildes (1929) has found that the period required for the germination of spores of *B. tetani* in a suitable medium at 38° depends mainly on the time required for this medium to reach a suitable reducing intensity. This reducing intensity was measured by Clark's indicators of oxidation-reduction potential. It was found within certain limits that *p*H except in so far as it affected the reducing intensity of the medium had no effect on the lag in growth. It was found that the greater the reducing intensity the shorter was the lag, and conversely the slighter the reducing intensity the longer the lag. A zone of reducing intensity is reached at which germination is not observed to occur at all. This effect of reduction potential is interesting in relation to the oxygen tension necessary for growth. It is probable that the germination of numerous spores is dependent on the setting up of a definite potential at which growth is possible. An account of the relation of reduction potential to growth will be found in the manual of Stephenson (1930).

Dormancy. Though in general under the optimal conditions of growth germination takes place in a relatively short time, some spores lie dormant for long periods before germination takes place. Thus Burke, Sprague and Barnes (1925) found that although the majority of *B. subtilis* spores develop in 4–5 days some may lie dormant for as long as 90 days. McCoy and Hastings (1928) found that spores of *Cl. acetobutylicum* and *Cl. Pasteuranium* in media all conducive to good growth lay dormant for periods of 11 to 222 days.

The germination time has been mentioned previously, but it is evident that it is difficult to decide the moments at which germination proper begins and ends. Swann (1924) investigated this in *B. anthracis*. The germination period was defined as the time between the beginning of incubation and the first division of the germinated cell. He found that this period is short (about 85 minutes at 37°), constant, and independent of the age of the spore up to 47 days, provided the culture be moist. The germination of old spores in dried cultures shows a minimum of 2 hours and a maximum of 7 hours.

An interesting observation has been made by Christian (1931) on a spore-forming organism isolated from commercial sterilised milk. It was found that if the spores were inoculated into milk and the spores heated germination invariably occurred. If to a small culture of heated spores possessing the power to germinate a small quantity of a living culture of the vegetative form was added, a number of the spores were so affected that they immediately lost the power of germination. If, however, to the spores which could not be induced to germinate by heating alone, a small quantity of a killed culture of the stable vegetative form was added, germination followed by normal spore formation took place. It appears that the stable vegetative form which has been found to dissociate from the sporulating form bears an inhibiting factor which is heat labile.

VI. THE RÔLE OF SPORES.

Before some interpretation of the significance of the process can be attempted it should be noted that in some species of bacteria which form endospores hereditary variants have been observed which do not form spores. Roux (1890) found that such a variant of the sporulating form of *B. anthracis* could be produced by growing in broth containing 1 in 5000 to 1 in 1600 phenol. The asporogenous form resembled the sporogenous very closely in the appearance of cultures. Its virulence was less than the original form, but it could be raised by passage through susceptible animals. Nevertheless it remained asporogenous while under observation. Eisenberg (1912) noted that this same organism produced non-spore-forming variants. He believed that all normal anthrax cultures were a mixture of sporogenous and asporogenous races. Rosenthal (1926) found that a similar asporogenous form of this organism could be produced by growth of the spore-producing form in filtrates of *B. anthracis* cultures. There does not appear to exist any relationship between the capacity of spore formation and virulence (Daranyi, 1927; Preisz, 1904). Asporogenous and sporogenous races may be equally virulent. It is interesting to note that Daranyi (1927) found that certain weakly sporulating strains investigated by him increased in sporulating capacity after mouse passage.

The rôle of spores in the multiplication of bacteria is uncertain. In most species only one endospore is produced per cell. Prazmowski noted that in exceptional cases *Cl. butyricum* developed two spores in a cell. Other instances are given by Migula (1904). Dobell (1908) noted that a giant bacillus found in the gut of frogs and toads formed two spores. In this form, *B. flexilis*, it appears, however, that the elongated double-sporulating element really consists of two cells that have not separated. Schaudinn (1902, 1903) noted a process in *B. bütschlii* which he interpreted as modified sexual reproduction (autogamy). Dobell (1908) agreed with this interpretation but later (1909) came to the conclusion that there was no evidence of a sexual process in disporic bacteria. The evidence appeared to be in favour of the sexual phenomena being due to a suppressed cell division. Not every cell produces a spore. It has been frequently observed that individual cells in a bacterial chain are sterile, although their neighbours produce spores. A good example of this behaviour was shown by Klein (1889) in *B. tumescens*.

No definite statement can be made as to the place of spores in the life cycle of bacteria. Thornton (1930) gives a brief but concise account of the present knowledge of the life cycles of bacteria. In two soil bacteria, *Azotobacter* and *B. radicicola*, there is definite evidence of the sudden appearance of new types. Reproduction may take place by buds or gonidia in these bacteria. There does not appear to be any evidence for a sexual process. Daranyi (1930) has claimed that the bacilli which are derived from a fresh spore are distinct from the older vegetative forms. He notes that they have a better growing capacity as evidenced by the growth of secondary colonies. He has noted also that cultures of *B. anthracis* left on moist agar for 2–3 days develop light patches on the surface of the agar. This "pseudobacteriophage" is found to be due to the fact that the young spores secrete a proteolytic enzyme which lyses the vegetative cells of the same species. This observation, which is of the greatest interest, will be discussed later.

Daranyi considers that "spore formation is the most primitive form of germ cell formation, whereby the bacterial cell is transformed completely into a single germ cell, which is followed by a rejuvenation without conjugation or fertilisation." This worker is also of the opinion that the ability of forms to spore depends in general on the same optimum conditions which lead to good vegetative development of the bacteria. Spore formation is brought about primarily by colloidal reactions. The most important of these is a diminution of the water content of the organism, resulting in a shrinkage of the colloids. Under natural conditions spore formation begins when the organisms grow older. This ageing is primarily due to loss of water on the part of the colloids. The lack of food material for the bacteria has a favourable influence on spore formation only in that the organisms get poorer in water. As noted previously, it was claimed that artificial dehydration brought about spore formation in young cultures of the organisms.

The resistance of the spores to high temperatures and drying has sometimes been attributed to the low water content (Lewith, 1890; Daranyi, *loc cit.*). There is unfortunately very little evidence as to the water content of bacterial spores. It has been already noted that in the spores of moulds the water content is reduced to a minimum. It is by no means improbable that this is also the case with bacterial spores. In considerations of this nature it is presumably assumed that the spores are completely homogeneous, but, as we have seen, there is quite definite evidence of a membrane which may be comparatively thick (see Brefeld's description of the process of spore germination in *B. subtilis*, p. 18). This fact would also explain the resistance to heat and to drying, rather better than an assumption that the spore is a homogeneous mass of low water content. It would also explain the greater resistance to disinfectants of spores as compared with vegetative forms. It appears also that the spore membrane of moulds is relatively thick and that there is in proportion a greater amount of membrane, and a less highly differentiated cell structure, than in the mycelial form. It might be expected that the membrane would be of low water content, thus accounting for the low percentage of water found by analysis of the whole spores. It is perhaps significant that Magoon (1926, 2) showed that the resistance to heat could be increased by a process of selection, and that the highest resistance was developed under conditions of moderate temperature and humidity. It is felt that experiments where the effect of dehydrating agents have been studied are not of much value unless they are correlated with the actual amount of growth observed. It seems also highly improbable that the complex cytological changes noted in the second section could be explained through one single physico-chemical effect.

It is quite evident from Section III that spore formation occurs while active growth is taking place. In an old culture of a spore-forming organism, however, the majority of cells present are found to be spores. This fact has no doubt been the cause of the explanatory statement that the spores are only formed when the medium becomes no longer suitable for the growth of the organism. A critical examination of the evidence shows that this explanation is quite fallacious. The marked proteolytic activity of the spores has been noted by Effront and Cook. Daranyi has shown that this proteolytic enzyme is capable of lysing the vegetative forms on an agar slope. There is little doubt that the spores are more resistant to these enzymes, although it is to be noted that if certain sugars are present, *e.g.* glucose, complete autolysis results (Cook, 1931). This fact is probably to be explained by the development of an acid reaction as a result of the bacterial activity which facilitates further autolytic processes to which the spores are not resistant.

Henrici (p. 11) noted that spore formation proceeded more rapidly on media of lower nutritive value and with heavier seedings. It appeared that spore formation is determined by the density of population in relation to the concentration of food stuff in the medium. Williams (p. 11) found that there was a higher ratio of spores to vegetative forms in 1 per cent. peptone than in 5 per cent. A greater production of proteolytic enzymes would be expected with the weaker nutrient material, thus accounting for the presence of the greater number of resistant spore forms. The results obtained by Michailowsky are probably explained by the fact that the addition of the lipoid solvents, which have no effect on proteolytic enzymes, kills the vegetative forms which are then lysed, and the spores appear to be the only cells present.

In regard to the fact that the spores are resistant to the proteolytic enzymes that are produced as a result of their own activity, it is interesting to note that certain other organisms form variants which are resistant to unfavourable conditions. Fleming and Allison (1927) noted that some resistant colonies of *M. lysodeiktus* were produced when this organism was grown in contact with lysozyme-containing substances such as tears and egg white. This property was inherited by these strains. Hydrogen peroxide is produced in the aerobic cultures of certain organisms and it ultimately causes the death of the bacteria. Todd (1930) has found that certain strains of haemolytic streptococci were formed which were resistant to its action.

The habitat of most if not all of the spore-forming bacteria is in the soil. It has been noted already that the spores are resistant to drying and other adverse conditions. The teleological significance would appear quite evident. It is found, however, that the majority of bacteria in the soil are not endospore formers. The following quotation is taken from Waksman (1927): "By far the greatest number of micro-organisms found in the soil by means of the microscope consists of minute non-sporing rods and cocci. The large spore-forming bacteria as *B. megatherium* and *B. cereus* have been found in normal soil only in the form of spores, which make up a very small proportion of the total bacterial flora of the soil." According to Winogradsky (1924) the spore-forming bacteria become active in the soil only when a great excess of easily decomposable organic matter has been added, or when the moisture content of the soil is high.

It is quite evident that other types of bacteria present in the soil are able to withstand an unfavourable environment for some time. The cocci and short rods and *Azotobacter* are largely connected with the colloidal soil particles in the form of zoogloea, surrounded by slimy capsules. The resistance of these forms is probably due to the presence of this capsule.

In the Chlamydobacteriaceae or thread-like bacteria, such as *Leptothrix* and *Crenothrix*, a thickening of the individual cell and a contraction of the cell giving coccus-like fragments or gonidia is observed. These forms were known by the older workers as Arthrospores.

The process observed here is somewhat like that noted in the encystment of protozoa in which a thick wall is secreted round the body of the organism. It is interesting to note that the formation of cysts has also been regarded as a direct response to unfavourable conditions, and excystation as occurring whenever a cyst is in an environment suitable for active life. It appears, however, that internal causes may play at least as great a part as external factors (Kofoid, 1923). In some yeasts a very similar process to that observed in endospore formation in bacteria occurs. The conditions governing this phenomenon are even less well known than with bacteria. References to this process are given by Guilliermond (1920).

It is perhaps significant that the majority of spore-forming bacteria are rather large forms and often form long threads. The colonies on solid nutrient media are of a rhizoid nature, *i.e.* growth of an irregular branched or root-like character, and not unlike that observed in the growth of certain moulds. It is possible that these forms have an affinity with the true fungi, especially as shown by the Mucorini. In these forms the spores are produced inside mother cells, the walls of which remain intact till the spores are ripe, forming a spore receptacle or sporangium. The spore-forming bacilli may be degenerate forms of these moulds or at least derived from the same parent stock.

There is no doubt that bacterial spores are not necessarily formed because conditions in the environment have become unfavourable for further growth. It is extremely likely that they represent a stage in the life cycle of certain bacteria. The difference in the metabolism between the spore and the vegetative form as noted by Cook (1931) is of interest in this connection. The respiration of the spores is less than that of the vegetative forms, but the proteolytic activity appears to be more marked.

It would be interesting to note if, on following the process of bacterial growth, the number of cell divisions could be correlated to the appearance of a spore. The formation of non-sporulating variants is of interest, as these forms are morphologically little different from the spore-forming varieties. There does not appear to have been any work done on conditions that will bring about the reappearance of spores in this form. The subject of bacterial variation is in a state of considerable flux, and further work on the morphological changes which occur during spore formation in relation to this phenomenon would be of great interest.

The function of spores is by no means clear, but they possibly represent a survival from a parent form. As these bodies are heat and drought resistant, they are undoubtedly in many instances a factor in the survival and distribution of the race and the only form in which certain adverse periods are passed through. For this reason, a rôle has been assigned to them. They are specifically produced to tide over unfavourable periods. It is true, as we have seen, that the process may be modified, but the process is characteristic of the organisms. Until the teleological attitude on the value of spores is discarded and spore formation is fully related to external factors, neither its value, if any, to the organism, the actual processes involved, nor its part in the life history can possibly be known. As to the significance of these forms either in bacteria or in moulds a more complete knowledge of the fundamental biological processes must be awaited. The only interpretation that can be given as yet is that they form spores because they form spores.

VII. SUMMARY.

An account is given of the process of spore formation in bacteria. Some of the cytological processes involved are described and the relation of these to the existence of nuclear structure in bacteria is discussed. An examination has been made of the evidence as to the effect of changes in environment on sporulation. The germination

of spores and the conditions under which it takes place are described. A contrast is made of the composition and metabolism of the spores as compared with the vegetative form of the organisms.

The conclusion is drawn that although spores are often a factor in the survival of the race in virtue of the fact that they are heat and drought resistant, they are not necessarily formed on the onset of unfavourable conditions, but are normally produced as part of the life cycle of certain bacteria. The process of spore formation in bacteria appears typically only in the Bacillaceae and it is suggested that the spores represent in these forms a survival from a parent mould-like form.

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"LA FIXITÉ DU MILIEU INTÉRIEUR EST LA CONDITION DE LA VIE LIBRE." (CLAUDE BERNARD)[1]

BY J. BARCROFT.

(*Received* August 1, 1931.)

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I. INTRODUCTION.

OF the principles which govern the physiological processes of the human body, that of the fixity of its internal environment has been as thoroughly established as any. Within the last twenty years works of first-rate importance by Haldane, Henderson and Cannon have all dealt with the subject. Great progress has been made in our understanding both of the mechanisms which secure the constancy of the internal medium and of the exactness with which these mechanisms operate.

The principle enunciated by Claude Bernard, if dressed up in modern language, seems to me just a little grotesque. To say that the temperature of the body is adjusted to the tenth part of 1 per cent. on the absolute scale, or the hydrogen-ion concentration of the blood to the hundredth part of a pH so that the organism may obtain a free life, is surely to make a very ill-balanced statement. The accuracy of the first clause contrasts almost comically with the vagueness of the second.

Indeed this "up-to-date" version of Claude Bernard's statement constitutes almost a challenge. In general, the "end" is more important than the "means," therefore it seems at least desirable to make some effort towards arriving at a conception of the liberty of life to be attained by the fixity of the *milieu intérieur*.

There are two obvious avenues of approach to the problem.

It is instructive to study the efficiency attained in forms of life humbler than those endowed with a circulating medium of constant properties, to see how nature has ensured some degree of efficiency up to that point, ascertaining if possible the mechanism which ensures constancy and from what that mechanism is developed.

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[1] The essential points in this article were given in one of a series of Dunham lectures in Boston, 1929, and later expanded into three lectures in London University.

The second avenue of attack is that of breaking down the constancy of the internal "environment," preferably in that form of life—man—in which it is most highly developed, and noting the nature and degree of impairment which takes place.

To commence with, then, we may select certain physical and chemical properties of the blood which are maintained at an approximately constant value; we may endeavour to ascertain how this constancy was attained; we may then proceed to consider at what disadvantage, if any, the organism was, while as yet the internal circulating medium was variable.

Lastly, a word as regards the phrase "milieu intérieur": I do not regard the specialised hormones, such as adrenalin, as part of the general environment, nor do I include drugs. I have included sugar, possibly illogically, but it is very interesting.

II. HYDROGEN-ION CONCENTRATION.

Every process of the living aerobic cell tends to alter the chemical composition of the medium in which the cell is situated in at least two ways. The mere fact of life tends to deprive the environment of oxygen and, if that environment be fluid, to increase its hydrogen-ion concentration. Not only is every cell in the body putting carbonic acid into the blood, but also the reaction of the circulating medium may be affected by special circumstances proper to the specialised activities of certain cells. Those of the pancreas when thrown into activity abstract alkali, and the oxyntic cells of the stomach abstract acid.

There is therefore every opportunity for the hydrogen-ion concentration of the internal environment to be inconstant. Yet in man it remains remarkably constant. The variation given in current books and taken from Van Slyke's figure (1921) is 7·0–7·8 *p*H, *i.e.* roughly 1–5 gm. in 10^8 litres—about a fivefold variation. It is unnecessary to stress the smallness of the absolute quantity, it is equivalent to 1–5 gm. of hydrogen spread over the total volume of plasma of all the people in the United Kingdom, or about half the people in the United States. Yet that is the variation for the extreme limits of human life, the variation as between fatal coma and fatal convulsions. The concentration of hydrogen ions in the plasma of a healthy person is regulated about five times as exactly.

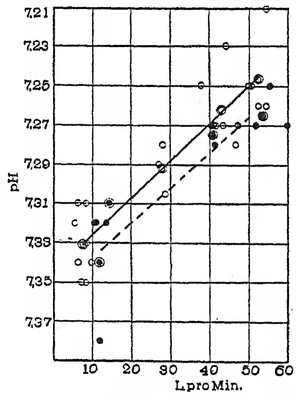

Fig. 1. Ordinate = reaction of blood, abscissa = ventilation in litres per minute; ○, individual observations on M. A.; ◎, average of observations on M. A.; ●, individual observations on G. L.; ⊙, average of observations on G. L.

The data are given graphically (Fig. 1) by Arborelius and Liljestrand (1923).

From rest up to work involving 50 litres per min. oxygen intake (945 kg. metres per min.), the alteration in hydrogen-ion concentration of the blood is given as from *p*H 7·33 ($c\text{H} = 4{\cdot}8 \times 10^{-8}$) to *p*H 7·26 ($c\text{H} = 5{\cdot}5 \times 10^{-8}$) for the average value obtained from determinations of the two authors.

Similar figures are given by Dill, Talbott and Edwards (1930) as follows:

Subject	*p*H		Change of *p*H	Ventilation per kg. of body weight (litres)
	Rest	Work (running 20 min.)		
D. B. D.	7·42	7·39	− 0·03	0·58
P. F. P.	7·44	7·32	− 0·12	0·80
A. A. McC.	7·42	7·37	− 0·05	0·72
J. H. T.	7·40	7·44	+ 0·04	0·67
W. C.	7·44	7·29	− 0·15	1·19
O. S. L.	7·41	7·31	− 0·10	0·67
J. L. S.	7·39	7·37	− 0·02	0·72
H. T. E.	7·39	7·30	− 0·09	0·59
A. V. B.	7·40	7·40	0·0	0·70
W. J. G.	7·36	7·38	+ 0·02	0·81
Average	7·41	7·36	− 0·05	0·75

The amount of work done was of the same order. The extreme case was that of W. C., who did work corresponding to a total ventilation of 82 litres per min. The alteration in the hydrogen-ion concentration of his blood was from $c\text{H} = 3{\cdot}6 \times 10^{-8}$ to $c\text{H} = 5{\cdot}2 \times 10^{-8}$, a proportional increase of 1 : 1·45.

Instead then of a possible alteration of 500 per cent. in the viable limits, the variation even in heavy exercise is only 45 per cent.

A very interesting point in the estimations of Dill, Talbott and Edwards is the range of normals. They lie between *p*H 7·36 ($c\text{H} = 4{\cdot}4 \times 10^{-8}$) and *p*H = 7·44 ($c\text{H} = 3{\cdot}6 \times 10^{-8}$), a variation of range of only 22 per cent. This range is scarcely greater than that of the maximal daily variation in a single individual as found by Cullen and Earle (1929). The blood may become more alkaline towards evening to the extent of seven-hundredths of a *p*H.

COMPARISON OF THE CONSTANCY IN MAN WITH THAT IN LOWER ANIMALS.

With these figures let us contrast those from the fish. Determinations, kindly given me by Dr Hall and Dr Gray of Duke University from the blood of the scup, taken at rest, showed a variation in the hydrogen-ion concentration of the blood between *c*H of 3×10^{-8} and of $2{\cdot}5 \times 10^{-7}$, *i.e.* 100 : 833, a variation not of 22 per cent. but of 800 per cent. The extreme limits of hydrogen-ion concentration in the blood of sub-mammalian forms is given differently by different authors and is quite obscure. Rohde (1920) believes that frogs normally have a *p*H which varies from 6·32–7·13, and that if fed on boric acid it may fall within 10 minutes to 4·2, while if fed on soda it will rise to 8, *i.e.* a ten-thousandfold variation. These figures, however, have not been substantiated by Mrs Hertwig-Hondru (1927), who placed the variation within much narrower limits (7·36–7·59). Even these are much larger variations than for man.

The following figures are given for the hydrogen-ion concentration in the insects cited (Glaser, 1925):

	*p*H	
Grasshopper	7·2	7·6
House-fly	7·2	7·6
Cockroach	7·5	8·0
Malacosoma	6·4	7·4
Bombyx mori	6·4	7·2
General range	6·4	8·0

The evolution of the whole mechanism which participates in the regulation of the hydrogen-ion concentration of the blood is a matter of extraordinary interest. It concerns primarily the kidney, the blood itself, and the respiratory centre.

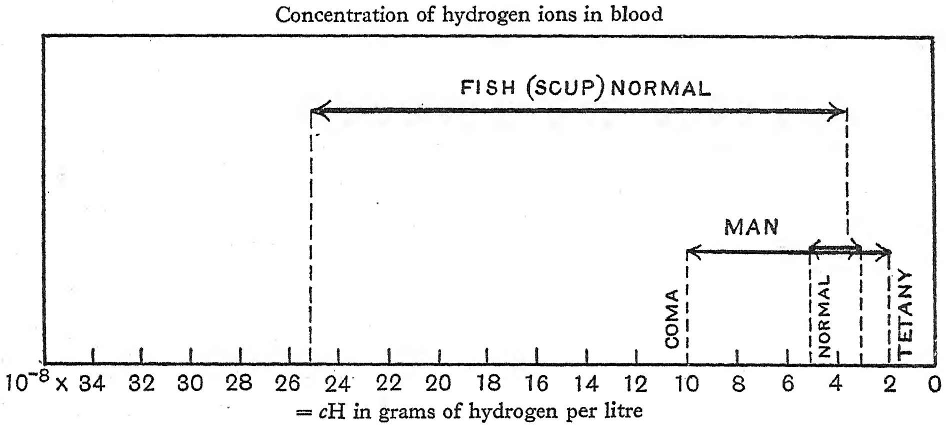

Fig. 2.

The blood. The evolution of the blood has been such that in general the more highly developed the form of life the less does the addition of a given quantity of acid or alkali alter the hydrogen-ion concentration, that is to say the more perfectly is the blood buffered. This is only true in a very rough sense. For this purpose the animal kingdom may be divided into great blocks: (1) the subvertebrate forms, (2) the cold-blooded vertebrates, and (3) the warm-blooded vertebrates, the birds and mammals.

Moreover there are three principal ways in which the addition of acid to the circulating fluid is prevented from suddenly and unduly altering the hydrogen-ion concentration of the same:

(1) By the buffering of the fluid and the tissues in contact therewith.

(2) By the excretion of acid or retention of alkali by the kidney (and the production of ammonia).

(3) By the excretion of carbonic acid by the lung.

It will at once strike the reader that the first method is not quite "on all fours" with the second and third; the first is rather a method for the mitigation or "evasion" of the effects of the addition of acid and alkali, than for the regulation of the number of hydrogen ions present. This distinction is one which I will refer to; here it is not of great importance, but please note it for future reference.

In the circulating fluid in such situations as the water vascular system of the sea urchin, there can be very little buffering (see fig. 3). In many of the submammalian forms the blood is buffered to quite an appreciable extent. This buffering is associated with the acquisition of some sort of pigment for the purpose of carrying oxygen, the two chief of which are haemoglobin and haemocyanin. Haemoglobin is of course found in considerable quantities in the blood of many worms, while haemocyanin is the prevalent respiratory pigment in the arthropods and molluscs.

It is not to be supposed that these pigments had originally any purpose in the blood other than that of carrying oxygen, if indeed they had any respiratory purpose whatever. But in each case it was desirable, in order that the pigment should be as useful as possible, that the oxygen should be capable of the most easy detachment in the situations in which it was most badly needed. Such situations would in the main be those in which carbonic acid or some other acid was likely to accumulate, and therefore it has come to pass that these two pigments have survived. The buffering action appears to be purely incidental, and indeed it is doubtful whether in such low forms of life there is any particular object in having the blood very highly buffered.

So far then as haemoglobin is concerned (and the evolution of the principal buffer is the evolution of haemoglobin), the consideration of buffering appears to have been somewhat of an afterthought, for I hear from my friend Dr Redfield that he has discovered in a certain worm a form of haemoglobin in which carbonic acid has no effect on the affinity of the pigment for oxygen.

Similarly, the most primitive form of haemocyanin, like its counterpart in the haemoglobin series, does not appear to have any value as a buffer. The oxygen affinity of the more complicated forms is affected by carbonic and other acids. But here there is a difference between haemoglobin and haemocyanin.

With the transition from the lower to the vertebrate forms came the next and indeed the final stage in the evolution of a highly buffered internal medium for the body. That stage was the enclosure of the haemoglobin in corpuscles. It is not that corpuscles which contain haemoglobin are unknown in lower forms of life, but putting on one side the exact phylogenetic status of *Amphioxus*—the vertebrate does, as the invertebrate did not, systematically and extensively use intracorpuscular haemoglobin as its oxygen carrier, and in so doing it "amplifies" the value of that pigment to a remarkable degree. The merit of corpuscles as amplifiers has been pointed out with such emphasis and explained with such thoroughness, that it would be superfluous for me to say much. Commencing with Hamburger and ending with the exposition given in the Henderson nomograms, the mechanism is set out. The corpuscle wall is permeable to acid and impermeable, or according to

J. Mellanby less permeable, to base; therefore when acid is added to the plasma it or its equivalent passes in large measure into the corpuscle, there to be dealt with by the haemoglobin-bicarbonate system; in so doing it leaves the reaction of the plasma relatively unchanged.

There is no record of haemocyanin in vertebrate blood, nor is there any record of its being contained in corpuscles. Why, we do not know. The mechanism by which haemoglobin is held in corpuscles is as yet unexplained. Possibly haemocyanin does not possess the chemical qualities which form the basis of such a system. It is known for instance that if the corpuscles of many animals be centrifuged until the mass becomes transparent, and if then any haemolytic agent be

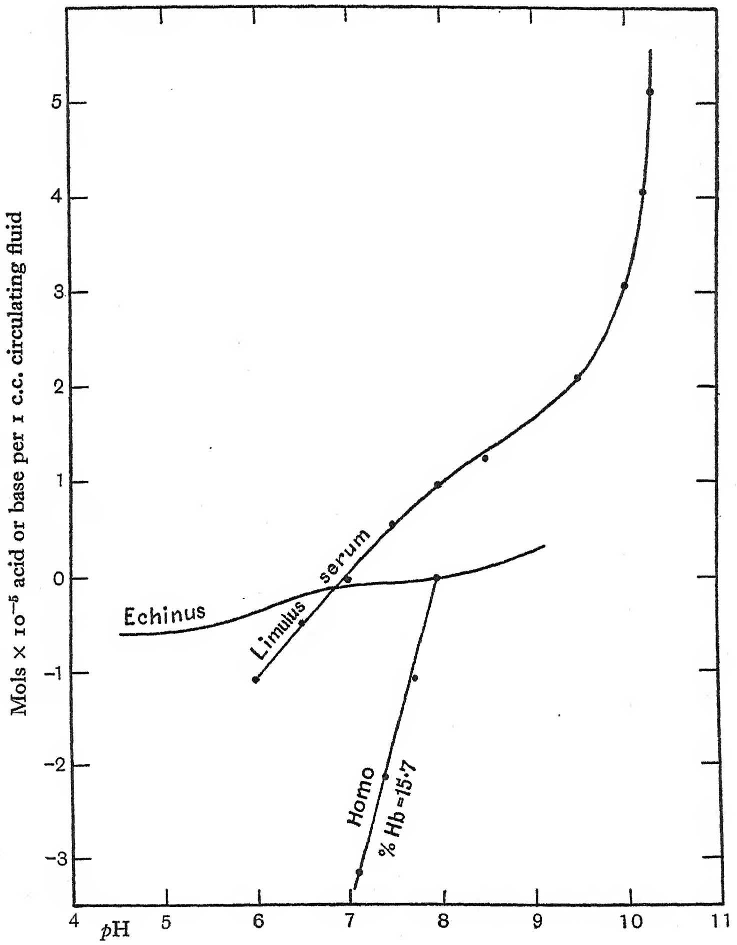

Fig. 3. Titration curves of blood of Man (after Terroux), of *Limulus* (after Redfield) and of *Echinus* (after Pantin).

added to the jelly, the haemoglobin will separate out at once in crystals. Yet it is clear from the nature of the dissociation curves that the haemoglobin behaves in the corpuscle as though it were in solution. According to Svedberg (1926) the molecular weight of haemocyanin is about 2,000,000, which would be about thirty times as great as that of haemoglobin. It appears to differ in different forms, being about 2,000,000 for *Limulus* and 5,000,000 for the snail. A molecule so gross may well be incapable of masquerading as a solution in concentrations in which it would ordinarily crystallise.

By the time we have reached the level of the lower vertebrates all the elements in the buffering of blood have been laid down. As regards buffering there is little difference in kind between frog's blood and that of an anaemic human being. The transition from the cold-blooded to the warm-blooded vertebrate is a matter of degree.

The preliminary step is that of producing an internal medium into which acid and alkali can be put with but a disproportionately small alteration in reaction; the finer points as regards the regulation of the hydrogen-ion concentration depend not upon the blood itself but upon the organs of excretion. As the excretion involves both substances in aqueous solution and carbonic acid gas the organs in question are the kidneys and the lungs respectively.

The kidney. The primitive organ for the regulation of hydrogen-ion concentration as of other things in the blood is the kidney.

I suppose the great problem which the kidney presents is: How can it regulate the blood concentration of so many substances simultaneously? Whatever the answer to this question may be, it would *a priori* not be surprising if in the effort to do a great many things at the same time, the kidney did each one of them a little less perfectly than would otherwise be the case. That may be the reason why, if the composition of the blood is altered by the injection of acid or alkali, a long time elapses before the previous *status quo* is entirely re-established.

Suppose there are 4·8 litres of blood in the body, and that the volume of blood which traverses the kidney is 2 c.c. per gm. per min., and the weight of the kidneys 320 gm., 640 c.c. of blood per minute will pass through these organs, or 13 per cent. of the blood in the body. Suppose (1) that a gram of some foreign substance, such as bicarbonate, is injected into the blood and only escapes therefrom in the kidney, and (2) that the blood leaving the kidney is entirely freed from this material, in 5 min. the amount in the blood will be halved, and in 20 min. there will only be about one-tenth of a gram remaining.

In point of fact the above assumptions do not hold. In general, material which can be eliminated by the kidney can also pass into the tissues, to be returned later to blood flow.

We may, however, "bracket" by making an assumption equally extravagant, namely that the alkali immediately diffuses all over the body, so that the whole body gets into equilibrium before any alkali is secreted; but let us retain the assumption that the kidney is as efficient as possible and that the blood emerged in the renal vein normal. On the above assumption, as the flow through the kidney per minute represents about 1 per cent. of the body weight (the water in that blood will be rather more than 1 per cent. of the water in the body) the excess of bicarbonate should be reduced to half in about an hour and to 10 per cent. of its amount in something of the order of 4 hours. One might expect then that the actual time for the elimination of alkali would be somewhere between that given by the assumption that all the alkali stayed in the blood and that all diffuses at once into the tissues and that in a time which was not less than 20 min. nor more than 4 hours the excess of alkali would be reduced to one-tenth of its original amount.

But in point of fact nothing of the kind occurs. Davies, Haldane and Kennaway (1920) showed that when a considerable amount of alkali (57½ gm. of sodium carbonate, within about 20 min.) was ingested, after 9 hours the kidney was still eliminating sodium carbonate in large quantities; after about 12 hours the elimination ceases.

It would seem that our second assumption must go too, as the kidney does not reduce the venous blood to normality and is therefore not as efficient as it might conceivably be.

The kidney apparently works—so far as alkali is concerned—on quite different principles. The authors quoted made the illuminating observation that at best the kidney produces (presumably because it can do no more) urine of a certain limiting concentration of alkali (about 2·5–2·8 per cent.). Any further increase in the elimination of alkali beyond that obtained by raising the content of the normal volume to 2·8 per cent. must be effected by increasing the elimination water. In that case either the concentration or the amount of every soluble substance in the body becomes affected indirectly. Short of knowing that the kidney cannot eliminate urine of more than a certain alkaline content we are very much in the dark as to any quantitative statement regarding the factors which regulate the elimination of alkali by the kidney. There seems, however, to be good reason for supposing that the influence of the nervous system does not figure prominently as one of them.

Many researches have of course been made upon the effect of the nervous system upon renal secretion, but none of them give information as to the rate at which a given excess of alkali or acid is rectified when the kidney is denervated. Margaria (1931), therefore, undertook such experiments. The general plan was that the kidney on one side was denervated by a preliminary operation; after a suitable time (10–45 days) the animal was rendered insensible either by chloralose, or in the later experiments by decerebration, cannulae were inserted into the ureters close to the bladder, and after sufficient measurements of the quantity and acidity of the urine from each kidney had been made, alkali was given, or in some cases carbonic acid. Taking the period before the actual administration of alkali, Margaria found, as others had done, that the excretion of water was more rapid on the denervated side; he found also that the excretion of acid was more rapid, too, but not in proportion to the rate of excretion of water. These effects were most marked in the experiments in which the kidney had been most recently denervated. In those in which a month or more had elapsed between the denervation and the final observations there was little difference between the two kidneys.

What has been said about the kidney has assumed that the function of that organ in relation to the regulation of hydrogen-ion concentration is one of selection; that it withdraws certain ingredients from the blood and at the same time eliminates them. No doubt in the main the picture is correct. Nevertheless the work, especially of Nash and Benedict (1921), has led to the belief that the kidney manufactures one very strong base, ammonia, out of a neutral substance, urea. Not only so but that it can throw the ammonia so made or some of it into the circulating fluid, so that the blood in the renal vein is richer in ammonia than that in the renal artery. There appears here to be a most interesting field for the comparative physiologist.

The lung. The activity of pulmonary respiration is of course conditioned by that of the respiratory centre. Our conception of the evolution and mechanism of the respiratory centre must at the present time be considered in the light of the work of Lumsden. His position may therefore be shortly stated. Lumsden (1923) postulates at least four centres situated at different levels in the brain. Of these the lowest is near the calamus scriptorius. It occupies the position of the classical "noeud vital." It is also phylogenetically the oldest. If I have followed Lumsden aright, he regards it as only a survival in vertebrates even as low as the tortoise. It is a gasping centre, and presumably even in the tortoise it must be regarded as an emergency mechanism. The interesting point about the gasping centre is its apparent unresponsiveness to CO_2. It appears solely to be actuated by want of oxygen. The animal takes in a gulp of air, and when the oxygen in this is exhausted it takes in another. In this connection, recollect that the main exchange of CO_2 in the frog is through the skin (Krogh (1904)), the function of the lung being primarily concerned with the acquisition of oxygen. Therefore the connection of the respiratory centre with regulation of a constant hydrogen-ion concentration is an afterthought; as in the case of haemoglobin the sequence of function is: oxygen intake, CO_2 output, regulation of constancy of hydrogen ions.

The next step in the evolution of the respiratory centre is typified by the brain of the tortoise. In it we have a response both to oxygen want, and CO_2 excess, but one which admits of rather considerable alterations in reaction. Want of oxygen increases the frequency of the rhythm, excessive CO_2 (30 per cent.) principally affects the amplitude, increasing the force of expiration. According to Lumsden (1924) respiration is operated in the Chelonian brain by three centres, each of which is situated in the medulla at the level of the fourth ventricle. Of these centres the principal is that which he calls the apneustic centre. Its activity consists in inflating the lung with air and keeping it so inflated. But (still following Lumsden's description) when the hydrogen-ion concen-

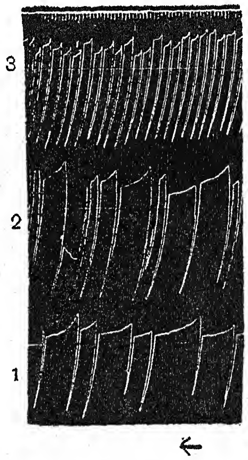

Fig. 4. Apneuses in the tortoise (after Lumsden). Upwards = inspiration, downwards = expiration. Reads from right to left. 1, normal; 2, breathing 30% CO_2; 3, breathing nitrogen.

tration in the blood (seen from Gesell's angle in the centre itself) reaches a certain limit, the inspiratory effort is broken down and a passive expiration takes place. This passive expiration over, the apneustic centre gets once more to work and another inspiration takes place. The second centre is an expiratory centre which comes into action simultaneously with the breakdown of apneusis and from the same cause. The centre augments the passive expiration, and that to a greater or less extent according to circumstances. Thirdly, there is the gasping centre which according to Lumsden is not ordinarily used.

As Lumsden points out, the arrangement which serves for the tortoise would be quite inadequate for the higher mammals. Among other things, each improvement in the buffering of the blood would tend to reduce its power to break down apneusis, and therefore the organism would tend towards a chronic condition of oxygen want. An additional mechanism has been therefore evolved which is found in the dog, cat and rabbit and presumably in higher mammals, a fresh centre has been added still higher up at the level of the upper part of the pons. Apneusis is now broken down by the action of the vagus on and through this higher "pneumotaxic" centre. As the stimulus to the vagus is regarded as the passage of air through the lung, caused by the very fact of apneusis, a type of respiration is effected in which the two phases follow immediately the one upon the other. Increased hydrogen-ion concentration in the blood continues to have a rôle, which is not to initiate one or another phase of the rhythm, but to regulate it—especially in the matter of depth.

The above description of the evolution of respiration is, as nearly as I can set forth, that of Lumsden. Let me now make some comments upon it.

First of all I wish to separate the actual phenomena which he describes from the rigid reference of these phenomena to precise anatomical centres. It seems to me that when a section affects a particular phase of respiration the deduction is no more than that some portion of the complex paths involved has been severed, and it is possible that if the animal were kept alive an alternative path might take its place. This criticism is put forward with great reserve from one who is not a neurologist.

Putting anatomical questions right out of the picture there remains the question in which I was much interested, whether Lumsden's physiological levels can be reproduced in ways other than sections. Of such methods I will mention two: the first is the respiration of hydrocyanic acid gas, the second is the study of foetal respiration.

A considerable research on the effect of HCN on the respiratory centre has recently been carried out by Taylor (1930, 1931). By the administration of air containing small quantities of the gas, the effects develop so slowly as to take 30 min., or a time of that order, to produce death. Moreover, they can be interrupted and reversed at any point. Lumsden's typical phenomena are the normal respiration, the apneusis and the gasp—in that order: at the commencement of poisoning the normal or pneumotaxic respiration turns into a series of apneuses, and these later turn into gasps. Any discussion of the transitional forms I defer for a moment.

The deduction from Taylor's experiments is that the gasp is the fundamental phenomenon of respiration. It appears to be unaffected by carbonic acid and therefore Taylor agrees with Lumsden that the fundamental movement proper to pulmonary respiration is not one which is prompted either by carbonic acid or hydrogen ions. Lumsden's observations evidently have a physiological significance and are not merely anatomical artifacts.

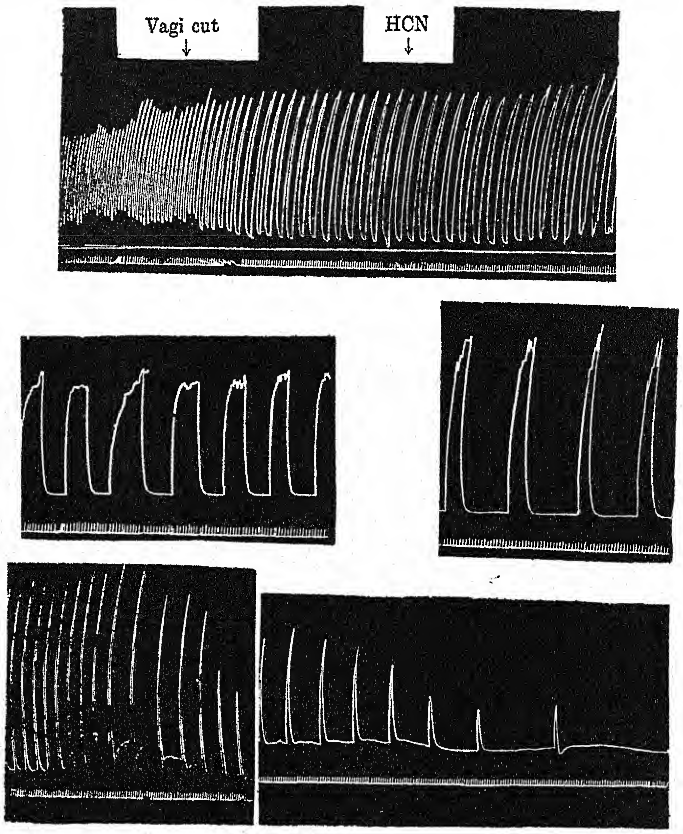

Fig. 5. Respiration of cat during continuous inhalation of air which contains HCN. (H. Taylor.)

At this point let me introduce the research of Huggett (1930). Huggett studied the physiological processes in the embryo. In foetal goats 2 months before their full term there are no respiratory movements; at least he was quite unable to confirm the work of Ahlfeld (1890) who, in opposition to Zuntz, held that such occurred. Starting then with the condition of quiescence, it was possible to induce single inspirations by one or other of two methods: (1) by stimulation of the central end of the sciatic nerve, and (2) as had previously been shown by Pflüger (1868) and Zuntz (1877), by clamping the umbilical cord. Cutting the vagus at this stage had no effect, as indeed might be expected in a condition in which the respiration was not of the pneumotaxic type. That stimulation of a sensory nerve such as the sciatic could institute respiratory movements in a quiescent respiratory centre is, however, a matter of great interest.

The nature of the gasp. Approaching this subject by three different avenues, those of brain section, cyanide poisoning and foetal development, we arrive at the "gasp" as being the fundamental phenomenon of respiration, and by two avenues, at the apneusis as being a sort of half-way house between ordered respiration and the gasp. It therefore seems worth while to enquire more particularly what may be the true nature both of the gasp and of the apneusis. In the first place the gasp seems unlikely "to have any previous history in the central nervous system," if I may use a phrase borrowed from Sir Charles Sherrington. In support of this contention three reasons may be given.

(1) In the marmot, during winter sleep, respirations take place at very infrequent intervals. The respiration when it does take place is complete of its kind, there is no delay between inspiration and expiration, but the inspirations occur at quite irregular intervals and perhaps 4 or 5 min. apart. It does not seem feasible to suppose that after the end of one respiration a reflex is drifting about the central nervous system for 5 min. before it prompts the next. Even if it did so the respirations might be expected more or less regularly at 5 min. intervals (Endres and Taylor, 1930).

(2) The same is true towards the end of the gasping period of cyanide poisoning. Each gasp is complete of its kind, fairly reproduces its predecessor and the expiratory phase follows the inspiratory phase with precision and without delay, but the gasps take place at rare intervals and irregularly. Obviously the causal relation between an inspiration and the subsequent deflation of the chest is quite different from, and of a much more intimate character than, the causal relation between expiration and the commencement of the succeeding gasp.

(3) The beautiful researches of Adrian and Buytendijk (1931) seem to indicate that in the goldfish and even in much lower forms of life respiration is a manifestation of an inherent rhythm in the central nervous system, and does not depend upon afferent influences which arrive from the periphery.

From the central nervous system, even after complete removal from the body, a rhythm may be tapped electrically and registered, which corresponds with that of the rate of movements of the gold-fish gills.

The gasp, apart from the property already discussed, namely that it is a separate entity, appears to have two others which may be noted here. It is maximal, involving probably all the respiratory muscles possibly in full degree, and it is very rapid.

For the reasons given the most legitimate way in which to consider the gasp (or at least its *nervous* mechanism) seems to me to be as a unit, which begins at the commencement of inspiration and which at the end of expiration is finished with. And now to study the gasp in greater detail, we may turn to the researches of Adrian, of H. Taylor and N. B. Taylor (1931). These researches consist in a study of

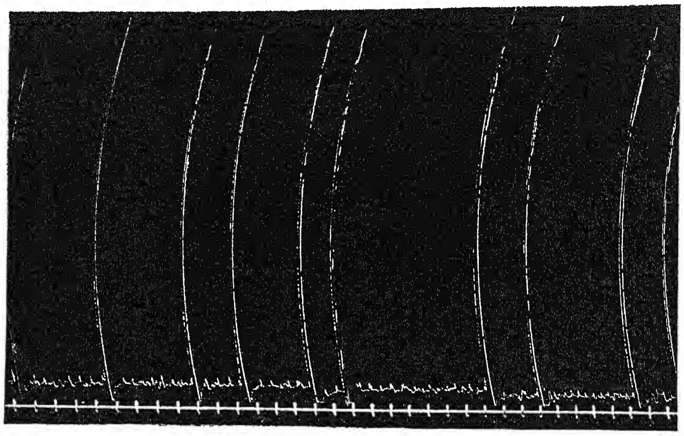

Fig. 6. Tracings of the volume of air inspired by the marmot. A rise of the lever denotes inspiration. The marmot was in an air-tight box, fitted with a spirometer, the movements of the lever of which were recorded. It breathed (through valves) air from outside the box. Time, ½ minutes. (By permission of the Royal Society.)

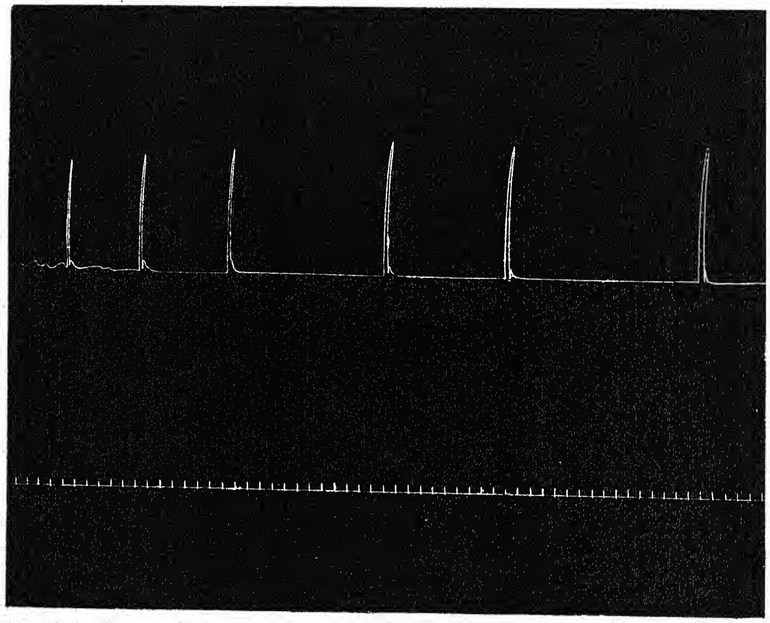

Fig. 7. Gasps in cat towards the end of cyanide poisoning.

the contractions of the muscles of expiration by the method of tapping the electrical variations which take place when they contract; the electrical variations are amplified and turned into sound waves. They can then be heard, so that by listening to the sounds emitted from a "loud speaker" an opinion can be formed as to whether in any particular phase of respiration the muscles of expiration are called into action. When this technique is applied to the triangularis sterni during gradual cyanide poisoning, we learn that as pneumotaxis passes into apneusis the triangularis sterni ceases to contract during expiration, and that during the periods both of apneusis and of gasping, costal expiration as an active process does not, or does not necessarily, occur. The deflation of the chest is purely passive. The abdominal muscles tell the same tale. We arrive at the conclusion, therefore, that a gasp is an inspiratory effort which untrammelled, passes over the whole, or nearly the whole, of the nervous centres actuating the muscles of inspiration—both those of normal inspiration and those of forced inspiration. Having traversed them rapidly "and without let or

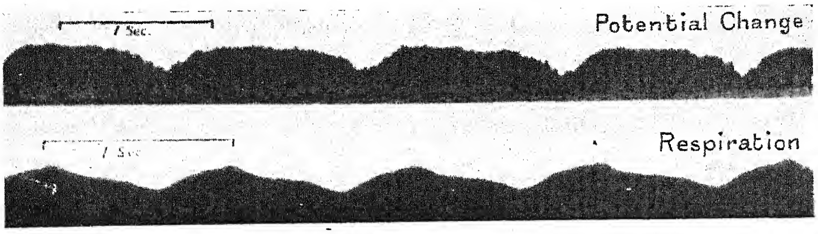

Fig. 8. Potential changes in excised central nervous system of goldfish compared with respiratory rhythm.

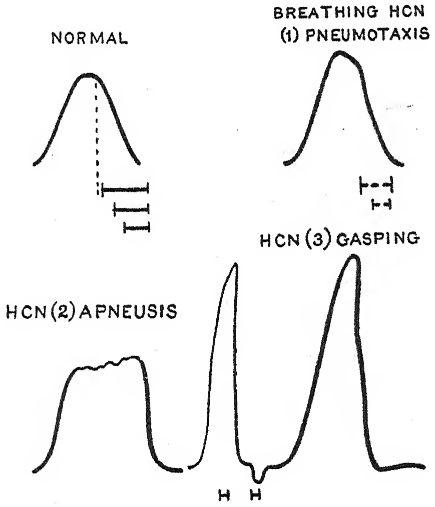

Fig. 9. Schematic representation of sounds heard in triangularis sterni muscle during expiration. ⊢⊣ = loud sound. ⊢⊣ = feeble sound.

hindrance" the wave is played out, the muscles concerned cease to contract and the chest becomes deflated.

Relation between the apneusis and the gasp. I have said, "without let or hindrance," and the phrase stands for more than a mere oratorical embellishment. There appear to be two distinct physiological processes involved in normal expiration, firstly the checking of inspiration and secondly the contraction of the expiratory muscles. Very often even in normal respiration they do not commence synchronously. In order of time the summit of the curve of respiration is signalised by the complete checking of inspiration (*i.e.* the relaxation of the inspiratory muscles), and the expiratory phase may be half over before the triangularis sterni or any other muscle which we have tapped commences to contract. Both these processes are abolished during the gasp, and the wave of inspiration goes "the whole way." Not only in a single respiration do the checking of inspiration and the appearance of active expiration not take place synchronously, but the latter process is abolished at an earlier stage in cyanide poisoning. In apneusis, the expiratory reflex has already been abolished, the inhibitory reflex is gradually being weakened. If a normal respiration be regarded as a modified gasp the process of inhibition must commence almost as soon as the inspiration itself. It is only necessary to superpose, as in Fig. 11, (1) a normal respiration, (2) an apneusis and (3) a gasp, taken from an animal which is "breathing" cyanide to see that the curve rises most slowly in the pneumotaxis and most rapidly in the gasp. Indeed on the tracing of apneusis actual breaks can be seen which correspond to checks in rate in the development of inspiration. In the case of apneusis, the inspiration is apparently inhibited at a certain point and a struggle takes place for a time between the progressing wave of inspiration and the presumably reflex inhibition which the wave brings into being; if the two are nicely balanced the apneusis lasts for a considerable period before the wave is spent and the chest deflated. It will be seen that, while we have confirmed Lumsden's phenomena, our interpretation of them differs from his in one important respect. He believes the gasping centre to be entirely superseded by the apneustic centre, we visualise an apneusis as being a modified gasp. That difference of opinion is based on information not at Lumsden's disposal, namely the gradual transition from apneusis to gasping when cyanide is administered, or from gasping

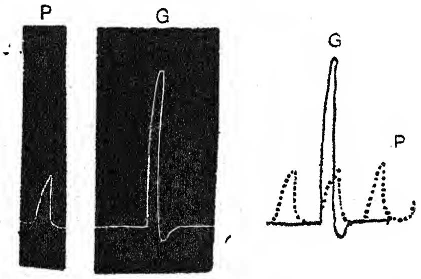

Fig. 10. Showing the relation (1) in amplitude, (2) in rapidity between the ordinary respiratory movements *P* and the gasp *G*, taken from the same tracing of cat inspiring air containing HCN.

to apneusis when cyanide is withdrawn. Fig. 12 shows this transition of the last few short apneuses alternating with gasps. It does not seem possible to regard the two as being due to the alternate workings of two different and independent centres. Such an idea would, I think, be very far fetched. The simple interpretation is that in the case of the apneusis the gasp is interrupted. The interruption is not so complete as at once to break down the inspiration; a struggle therefore ensues for a short time in which the inspiration spends itself in competing with the inhibition.

I am indebted to Adrian for pointing out to me that though at first sight it might appear unlikely that the inhibition should do other than completely abolish the positive phase of the inspiration, there is perhaps an interesting analogy in the work of Bethe (1930) and Bethe and Woitas (1930), who give numerous instances from beetles and other forms in which a simple purposive movement thwarted along the direct and usual line of attainment is not stopped, but persists to achieve its end by some unusual path.

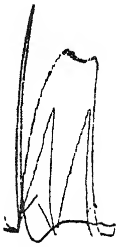

Fig. 11

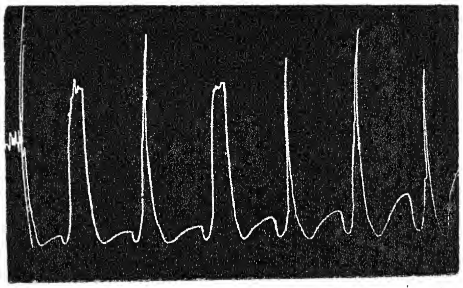

Fig. 12.

Fig. 11. Normal respiration, apneusis and gasp superposed.
Fig. 12. Apneuses passing into gasps. HCN. (Taylor.)

The relation of pneumotaxis to the apneusis and the gasp. The logical inference from the above argument is that, given a much greater degree of inhibition, the gasp would be reduced to a pneumotaxic respiration. So far so good, but I have said above with regard to the gasp "the inspiration spends itself in competing with the inhibition." But if the inhibition is increased to the point of suppressing the inspiration, the questions may reasonably be asked, Will the inspiration spend itself completely, and if not what will happen when the inhibition is removed? Perhaps I can picture the matter in the following way: suppose a gasp to result from 100 explosions in each of 100 cells in this centre, after which the cells rest till the system is re-established, and supposing in pneumotaxis only 30 explosions per cell take place and fewer cells are involved, say 50, then 1500 explosions only will have occurred and most of the explosive material will be left ready to go off as soon as the inhibition is removed. Will they commence spontaneously to do so? If so, a new and much more rapid rhythm will at once be established and that actually occurs.

In the light of more recent additions to knowledge we arrive at a conception of respiration which is a little different from that of Lumsden. A respiration commences with a wave of activity in the centre responsible for inspiration. This inspiration has all the potentialities of a gasp, but at once induces an effort to smother itself and it is broken down. Firstly there is inhibition of inspiration and secondly active expiration. Cyanide abolishes both these, but it abolishes the latter before the former. When both are gone respiration is reduced to its simplest and most primitive terms, namely a series of gasps. When the inhibition is reduced to very feeble proportions the respiration conforms more or less to the apneustic type.

The action of carbonic acid. Now to turn to the action of carbonic acid. As we have already said, CO_2 appears to have no augmentor effect at the gasping stage. How could it? Three possible ways suggest themselves:

(1) It might increase the inspiratory efforts, but it cannot effect anything in that direction because they are more or less maximal in any case.

(2) Were expiration an active process CO_2 might affect it, but in the case of the gasp, expiration being purely negative, there is nothing to affect.

(3) Though it cannot exert much influence on the nature of the gasp it might still reduce the time which elapses between the gasps. This it appears not to do. Here then is one perfectly definite piece of information, which though negative in character is well worth noting, because we can trace the characteristic of CO_2 right up to man. Short of some increase of CO_2 of the order of 5 per cent. in the inspired air it does not necessarily quicken respiration. In some persons CO_2 quickens respiration slightly, in some it has the opposite effect, in yet others it has no effect at all.

Haldane and Priestley (1905) found this in their classic investigation on the effect of CO_2 on the respiratory centre, and it has been the experience so far, I know, of others who have interested themselves in the matter. When we speak of carbonic acid stimulating the respiratory centre we mean or should mean that CO_2 (within ordinary limits) increases the amplitude immediately and the frequency ultimately. This is perhaps worth a word of emphasis because one constantly hears persons discuss the effect of CO_2 as being quickening of the respiratory rhythm (see Barcroft and Margaria (1931)). The introduction of the factors which cut "gasping" down to pneumotaxis provides the possibility of a new milieu in which CO_2 can work. Fig. 13 shows that in the gasping phase of cyanide poisoning the total ventilation is much greater than in the phase of pneumotaxis.

In the hibernating marmot the effect of CO_2 seems to be rather simple (Endres and Taylor (1930)).

(1) In the first place it postpones the crisis which turns inspiration into expiration. The curve *B* in Fig. 14 (marmot breathing air + 10 per cent. CO_2) rises no more steeply than curve *A* (marmot breathing air), but it proceeds further before it is checked.

(2) The expiratory phase, especially the latter part of it, is much accentuated. The respiration is complete when breathing CO_2 before the normal.

In man the effect of CO_2 is not so simple. Fig. 15 shows tracings taken by rebreathing into a Krogh's spirometer; the modifications which result are due to the breathing of the carbonic acid in the concentrations stated in the legend.

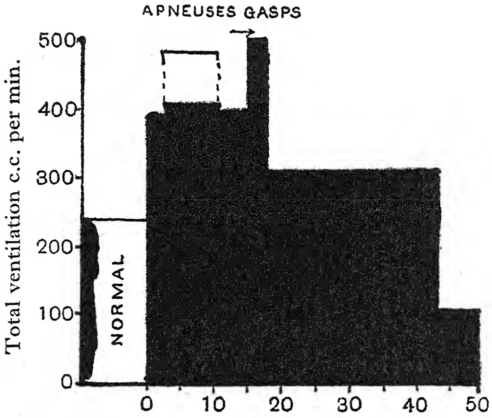

Fig. 13. Minutes during which HCN 0·31 g./m.^{3} is administered.

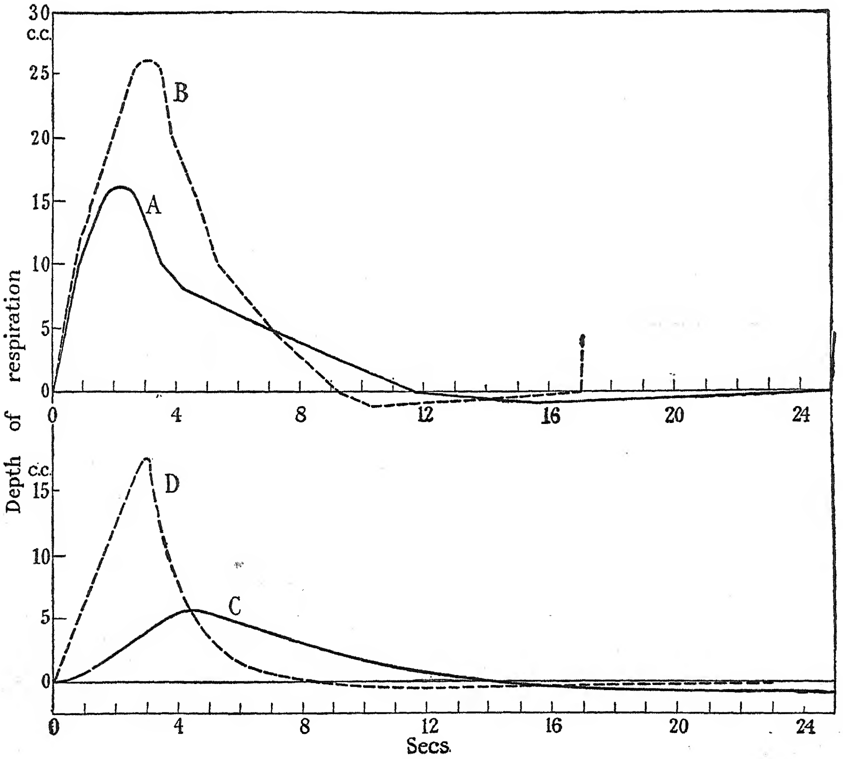

Fig. 14. A tracing of the volume of air breathed by hibernating marmots. The animal used for *A* and *B* was not the same as that used for *C* and *D*. Tracing *C* reaches the base-line at 45 seconds from the commencement. Temperature of marmot in *C*, 4·8°; in *D*, 19·6°. (By permission of the Royal Society.)

In man the curve of inspiration is much steepened by carbonic acid. That new factor, which provides some justification for the statement that "CO_2 stimulates the respiratory centre," demands a closer enquiry into the effect of CO_2 on the muscular contractions involved in respiration.

The effect of CO_2 on the diaphragm. In the central part of the diaphragm during normal respiration there is a condition of tone throughout the whole of respiration, heightened of course during inspiration and reduced during expiration. It can easily be appreciated by the Adrian technique; indeed I possess a gramophone record, made under Adrian's instructions by the Columbia Company, in which the muscular contractions in some parts of the diaphragm can be heard during the whole of normal respiration. The sounds wax during inspiration and wane during expiration.

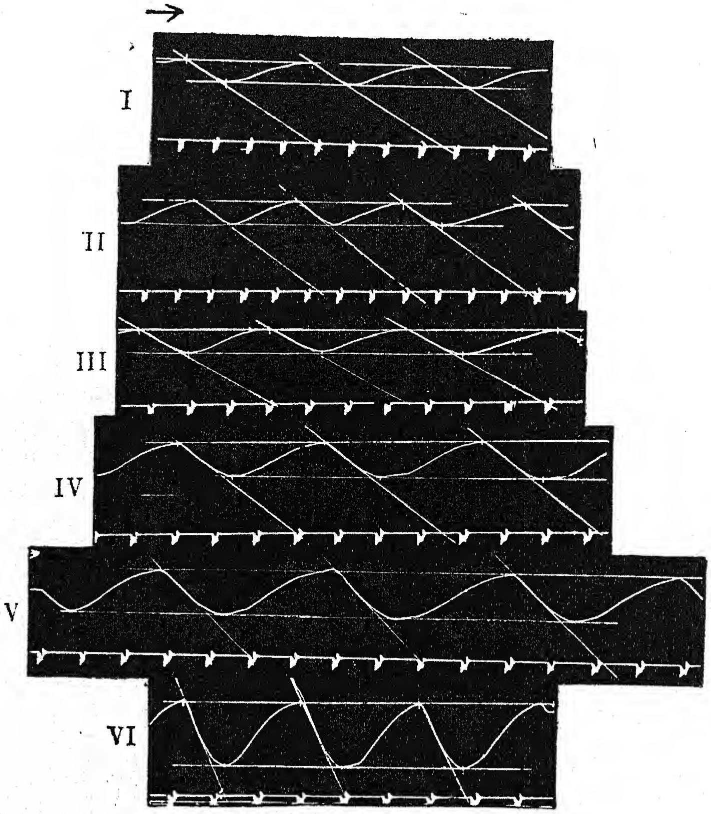

Fig. 15. Spirometer tracings of the respiration. Subject, Barcroft. CO_2 in inspired air: (i) 0·2 per cent., (ii) 1·0 per cent., (iii) 2·2 per cent., (iv) 4·2 per cent., (v) 5.3 per cent., (vi) 7·5 per cent. Inspiration downwards, expiration upwards. Tracing read from left to right. Time, 1 sec.

When the animal "rebreathes," the sounds become very much accentuated during inspiration and abolished during expiration. Presumably, therefore, the amplitude of the movements of the diaphragm increase in both directions[1].

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[1] This record was "exhibited" at the Dunham lecture in Boston in October 1929.

The effort to visualise what takes place during diaphragmatic respiration is perhaps allowable, even with no more information than we possess.

Let us start with a diaphragm in a state of partial tone; connected with it is a cell *R*, Fig. 16 (one of a great number of such), in the gasping centre. (I omit connections at the level of the phrenic nucleus.) Were there no more machinery the respiration would be of the gasping type, regular in rhythm, the frequency being that of the cell *R*. The total ventilation would be much greater than that of normal respiration. The gasping centre left to itself would produce hopeless over-ventilation. Too many cells, in fact probably all the cells (for gasps seem to be maximal), would "fire off" at the same time or in quick succession. It must be inhibited, and this inhibition is excited from above by some mechanism the activity of which is (1) impaired by section of the so-called pneumotaxic centre, (2) destroyed by HCN, (3) relatively undeveloped in the foetus, (4) heightened by the stimulation of sensory nerves.

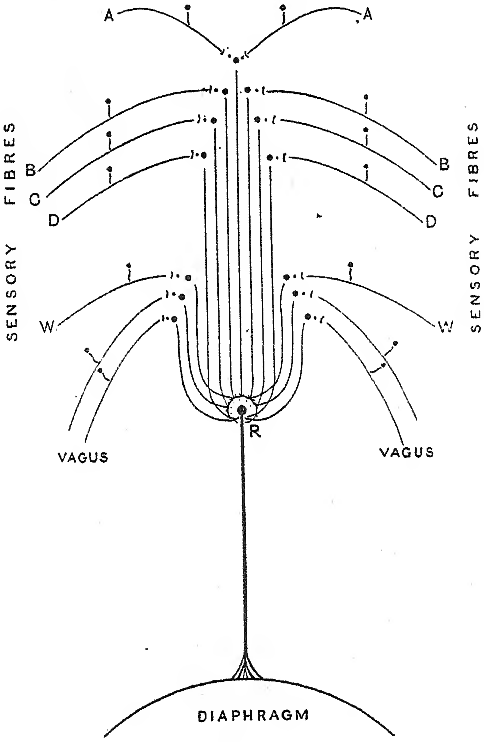

Fig. 16.

Inspiration once it is initiated evokes 10,000 (figuratively, probably more actually) sensory stimuli which immediately tend to throttle it. The relevant places from which they come are probably the muscles involved in respiration and the respiratory tract from the nose downwards to the lungs, thus involving primarily the fifth and tenth nerves.

These impulses are inhibitory in character and vary in degree with the force of the inspiration, thus a shallow inspiration will reduce the tone of the diaphragm during expiration, a deep one will abolish it. In any case they are powerful enough to account for the relaxation of the diaphragm during expiration. Probably only a few of these innumerable reflex stimuli are sufficient to preserve respiration, at all events a great number of avenues of sensation can be cut away without any great modification of the respiratory ebb and flow. The influences coming along the vagus alone are probably sufficient. Moreover, the connections of the vagus are probably relatively low down, for apneusis cannot be obtained if the vagi are intact.

But if the vagus be cut and if the impulses from above its point of entry are also cut off, the remaining afferent impulses are insufficient to enforce the breakdown of an inspiration, and the phenomenon of apneusis in which the inspiratory effort is checked, but not broken, is seen. If the section goes below the "apneustic centre" these last remaining sensory reflexes disappear, or at least they get reduced to a point at which they become ineffective and gasping will take place. Now consider what will happen on the above scheme when HCN is administered. On the theory put forward by Evans (1919), that HCN acts from above downwards, cells above *R* will be the first to be affected. As the finger is gradually removed from the throttle so the activities of *R* will become less and less restrained, the amplitude of the respiration will increase and hyperventilation will, as indeed it does, take place. This is the first phase of HCN poisoning, the so-called "stimulation" of respiration. But from our standpoint it is not a stimulation in any true sense, it is the removal of an inhibition. It is very difficult to think of HCN really as "stimulating" anything: it is a general protoplasmic poison. It is unnecessary to detail the other phenomena, they would happen in time just as Taylor found them to do.

Costal Respiration. The essential difference between diaphragmatic and costal respiration lies in the fact that the diaphragmatic muscles are purely inspiratory. The costal muscles are both inspiratory and expiratory. Concerning expiration we know but little. That there must be an expiratory centre is, I imagine, agreed by everyone now. That the expiratory centre is thrown into activity as the result of inspiration is also, I think, clear. In HCN poisoning the electric variations of the expiratory muscles are occasionally very well marked just after the gasp is over, so that the impression is given of an inspiratory gasp followed by an expiratory one. How far it is possible to apply to the expiratory centre the same arguments with regard to inhibition that we have to the inspiratory centre it is difficult to say. We can easily get inspiration without active expiration, we do not get active expiration without inspiration—in mammals at all events.

If the hypothesis that CO_2 acted like making suitable cuts through the medulla or like the administration of HCN were true, there seemed to be just a chance that the giving of enough CO_2 might produce the chain of symptoms, hyperpnoea, apneusis and gasping, which is characteristic of the other procedures. The event proved more successful than we had expected. Naturally if you set out to imitate HCN with CO_2 there is no use adopting half measures. So we gave the cat a mixture of 64 per cent. CO_2 and 28 per cent. oxygen, with the result that hyperpnoea at once supervened, to be followed by an obvious tendency to respiration of an apneustic type, this in turn passed into "gasping." There are differences in detail from HCN and also from the types as given by Lumsden, but it must be remembered that the action of HCN and CO_2 is at best differential. Their action on the higher portions of the brain is not complete before that on the lower parts commences. Indeed an attempt to obtain the whole chain of events produced (*a*) from sections, and (*b*) from HCN, by the administration of CO_2, proved to be much more successful.

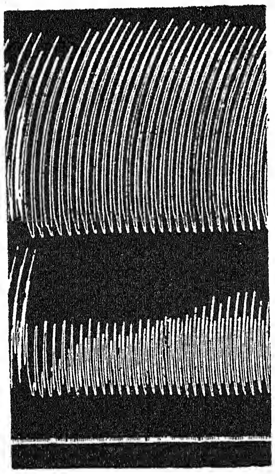

Fig. 17.

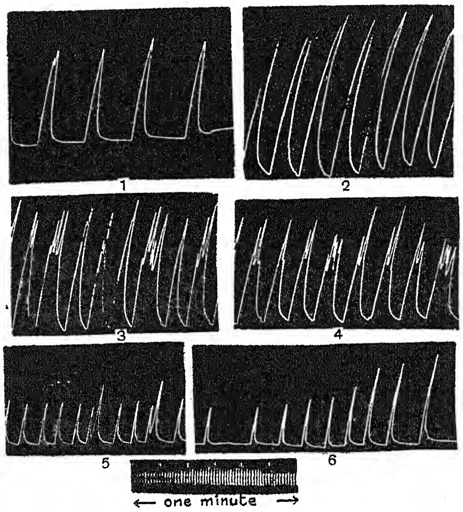

Fig. 18.

Fig. 17. Double abdominal movements (after pumping air) (cat). Top: thorax. Bottom: abdomen. Vagi cut. Time in sec. HCN = 0·28 gm. per cu. metre. The interpolated movements on the abdominal curve are caused by active expirations, the abdomen being forced outwards by the contractions of the thorax.

Fig. 18. Effect of CO_2 64 % on respiration of cat.

1, Normal	3, Apneusis	5, Gasping (imperfect)
2, Hyperpnœa	4, Apneusis (later)	6, Gasping

The important phenomena of diaphragmatic respiration have, I think, been accounted for with one exception, namely, that the rhythm is considerably faster than the natural gasping rhythm, which presumably is that proper to cell *R*. To that I will revert after saying a word about costal respiration. Accepting the general scheme of respiration as put forward above there is an alternative explanation of the action of CO_2 as was pointed out to me by Dr Margaria. It is as follows: carbonic acid stimulates at the level of *R*. During gasping the stimulus effects nothing because the gasps are maximal, but when *R* is subject to the normal inhibitions which play upon it, CO_2 causes it to "put up" a stronger fight against these inhibitions. This conception introduces the extra complication of taking CO_2 out of line with other things (HCN, etc.). Moreover there is something on the positive side to be said for the idea that the effect of CO_2 is rather to remove inhibitions than to cause active stimulation.

According to Heymans (1931) the respiration is easily affected by influences which reach the brain from the carotid sinus. When the carotid sinus of a dog *B*, and that only, be perfused with blood from a dog *A*, the respiration of *B* will be increased in depth and rate:

(1) if the blood pressure of dog *A* be lowered;
(2) if dog *A* breathes HCN;
(3) if dog *A* breathes CO_2;

whilst respiration in dog *B* is made more shallow and more slow:

(4) if the blood pressure in dog *A* be raised;
(5) if CO_2 be removed from the blood of dog *A*.

As between (1) and (4), the raising of the blood pressure is more likely to be a stimulus than the lowering of blood pressure—a negative act; and with regard to (3) it is much more easy to conceive of CO_2 as paralysing a nervous process than as stimulating it. The discharge in the cardiac depressor increases enormously with the blood pressure and presumably the nerve endings in the carotid sinus are much like those in the aorta. We have never tried CO_2 in the cardiac depressor discharge. On the whole, therefore, it is simplest to regard the tone maintained by the carotid sinus as an inhibitory one, the inhibition being lifted by procedures (1), (2) and (3) and increased by (4) and (5).

Rhythm. And so I come to the subject of rhythm, about which I feel that I have very little to say.

I have given reasons for supposing the gasping rhythm to be automatic, and I have shown how such a rhythm may be prolonged into a series of apneuses.

The ordinary pneumotaxic rhythm is clearly much more rapid than the gasping rhythm, and I have indicated one way in which such a rhythm might be produced, without very much in the way of new assumptions. In particular I have no explanation to offer of the effect of CO_2 on the rate of respiration, which effect is, as I have said, very inconstant.

Another way, but a way which requires one more assumption, is that the cells responsible for the inhibition are themselves rhythmic, and that the normal rhythm is that of the inhibitions which play upon a gasping centre which always has something in hand and is therefore never permitted to unmask its own rhythm. Here a very pretty analogy could be drawn with the cardiac rhythm, but I hesitate to draw it because after all it is only an analogy, the cardiac mechanism not being of the same specialised nervous nature as the neurones which compose the central nervous system. Indeed, I have perhaps strayed too far into theory or rather speculation. Let me therefore conclude with the enumeration of the specific points which I should like to stress.

Firstly, there are two methods of regulating constancy of the hydrogen-ion concentration, that of evasion and that of correction.

Secondly, the former of these is perfected, in kind, though not in degree, by the time the lower vertebrates are reached.

Thirdly, the latter is common to the whole animal kingdom; but

Fourthly, its most delicate mechanism only develops at the mammalian (and perhaps collaterally at the avian) level.

Fifthly, that most delicate regulation is a regulation by the nervous system, and by a level of the nervous system which is probably higher than the ordinary medullary centres.

III. TEMPERATURE.

Of the conditions which may be investigated the most attractive perhaps is a physical one, namely temperature, and therefore it will be considered in greatest detail. The temperature of man is approximately constant; how did it become so, and with what degree of success did creatures of inconstant temperature perform their bodily functions such as respiration, muscular contraction, heart beat, etc.?

Before these functions are considered in detail a few words may be said in general terms on the implications of alterations of temperature. From the physico-chemical point of view, the activities of the body consist of a vast number of chemical reactions which take place simultaneously. The final result depends upon a complete proportional and quantitative harmony of the rates at which these innumerable chemical reactions are proceeding.

Yet each reaction is a purely quantitative affair and is governed by the general laws of thermodynamics. Each, if it is reversible, is governed by the principle expressed in the equation of Arrhenius, namely that if the velocities at any two temperatures T_1 and T_2 are respectively K_1 and K_2, and if μ is the molecular heat of formation,

$$K_1 = K_2 e^{\left(\frac{\mu}{2} \times \frac{T_2 - T_1}{T_2 T_1}\right)},$$

the graphical implication of which is that if over a range of temperatures the logarithms of the velocities be plotted as the ordinate, and the reciprocals of the absolute temperatures (on the Kelvin scale) be plotted as the abscissa, the relationship appears as a straight line.

Reactions take place in the body which are not usually regarded as simple, to which the Arrhenius equation still applies. In this connection the equilibrium of haemoglobin with oxygen has been studied with great care. Twenty years ago that reaction was regarded as simple, now it is held to involve the association of four molecules of oxygen with each molecule (mol. wt. 68,000) of haemoglobin as well as the dissociation of the haemoglobin and even the extent to which it is united with sodium. Yet the greater the care with which the determinations are made the more convincing is the rectilinear relation between the logarithm of the 50 per cent. saturation pressure of oxygen and the reciprocal of the absolute temperature. Fig. 19 shows the results of recent observations by Goldschmidt and Ray (1931) on the subject. These observations were made upon solutions of haemoglobin of the ox. They are particularly convincing for the following reason. From the line obtained it is possible to calculate the value of the heat of formation of oxyhaemoglobin; on the same solution the heat of formation was actually measured by Roughton, and it agreed very closely with the calculated value.

Not only does the Arrhenius equation apply to haemoglobin solutions (Fig. 19) and to the equilibrium between oxygen and blood corpuscles at a constant hydrogen-ion concentration, but, and this is more remarkable, it applies to the equilibrium between oxygen and blood at a constant CO_2 pressure of 40 mm. of mercury, for the constancy of the CO_2 pressure at different temperatures presumably argues an inconstancy of hydrogen-ion concentration in the fluid. Carbonic acid is less soluble as the temperature rises. The former result was found on the corpuscles of the marmot by Endres (1930) (Fig. 20), the latter on its blood, in which he confirmed the results of Brown and Hill (1923) on human blood (Fig. 21). Thus reactions can become rather complicated without departing from the dominion of the Arrhenius equation.

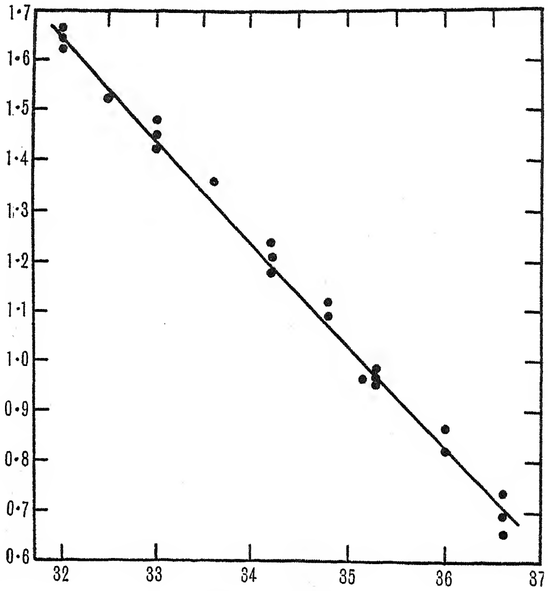

Fig 19. Ordinate, log of O_2 pressure at which haemoglobin is 50 % saturated. Abscissa, Reciprocal of absolute temperature × 10,000.

When account is taken of the fact that the velocity of each chemical reaction in the body is simply a property of itself, it is surprising enough that at any one temperature all the reactions of the body should progress at velocities suitable to one another. But it is much more surprising, nay it would seem well nigh impossible that, granting that the body should function at some one temperature, it should also function over a great range. For if the velocity of some one reaction got out of step with its neighbours the whole machine would jam—to use a phrase which I once heard from the lips of A. V. Hill.

On a casual scrutiny, then, nothing would appear more plausible than to give as a reason for the preservation of a constant temperature, the probable chaos which would result from any considerable thermometric alteration—to say, in short, alter

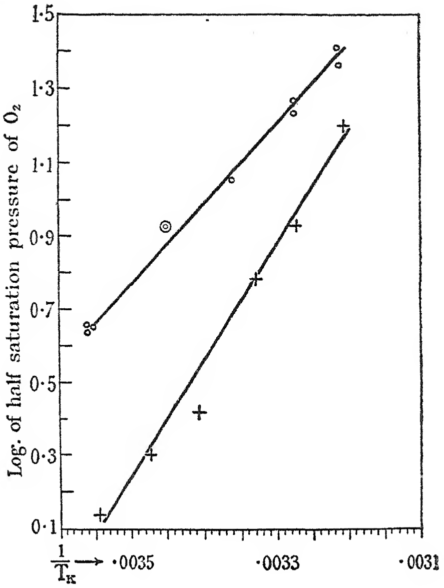

Fig. 20. Ordinate = log. of pressure at which the haemoglobin in the corpuscles is 50 per cent. saturated with oxygen. Abscissa reciprocal of absolute temperature. Upper line = corpuscles exposed to 40 mm. CO_2 pressure. Lower line = *p*H 7·76. (By permission of the Royal Society.)

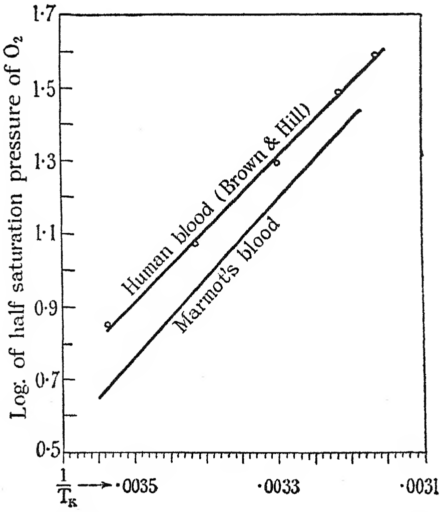

Fig. 21. Ordinate and abscissa as in Fig. 20. Upper line = human blood calculated from Brown and Hill (1923). The point corresponding to Brown and Hill's curve at 0° C. has been omitted because the half saturation pressure cannot be read with sufficient accuracy from their figure. CO_2 pressure = 40 mm. (By permission of the Royal Society.)

the temperature and the velocities of the reactions which form the physical basis of life must get out of gear.

Yet in reality enquiry shows to what a remarkable extent nature has contrived in one way or another to circumvent any such barrier imposed by this simple application of chemical laws.

How does the body of the animal of variable temperature so control its reactions as to keep them in step over a great range of temperature? In point of fact the poikilotherm seems to have devised methods of overruling to some extent the apparent consequences of the law of logarithmic increase in the rate of velocity of chemical action with temperature. Once we invade the region of living processes there are departures from such a simple rectilinear relation as that shown for the temperature coefficient of the haemoglobin-oxygen equilibrium. Krogh (1916) drew attention to the fact that the metabolism of the body as a whole did not in cold-blooded animals follow the logarithmic law, but fell off relatively to the requirements of that law as the temperature rose. And what is true of the body as a whole seems to be true of such of its individual functions as have been studied. A number of such specialised functions have been collected by Clark (1927), the rate of ciliary movement in *Mytilus* (Gray, 1923), the rate of movement of amoebae (Pantin, 1924), the frequency of the isolated frog's heart (Clark).

Of these, the temperature coefficients over a range of 10° C. do not remain uniform as the temperature rises, but tend to decrease as the temperature increases thus:

Temperature coefficients (Q_{10}) *of processes in invertebrate tissues.*

Temperature ° C.	Frequency of frog's heart	Rate of movement of *Amoebae*	Rate of movement and O_2 consumption of cilia of *Mytilus*
0–10	3·5	7·33	3·1
5–15	2·8	2·71	2·7
10–20	2·4	2·17	2·3
15–25	2·1	—	2·15
20–30	1·8	—	1·95

The above observations have been plotted by Clark; the frequencies in each case being expressed as percentages of that at 20° C.

A closer comparison of some such curves as are shown in Fig. 22 with the data from which they have been drawn leads to considerable doubt as to whether the points observed fall in reality upon a smooth curve. The greater the pains taken to secure accuracy of observation, and the greater the accuracy obtained and the more numerous the observations, the greater becomes the difficulty in drawing satisfactorily any sort of smooth curve through the plotted points which correlate the logarithm of the degree of activity of a vital process with the reciprocal of the absolute temperature at which it takes place. On the other hand Crozier (1924) and his school regard the essential character of such a figure not as being a smooth curve, but as being made up of two or more straight lines, each of which represents faithfully a chemical reaction. Of six hundred sets of observations which Crozier has made the majority fall into a simple scheme which involves two chemical reactions, each of which can be represented by a straight line, the lines being disposed more or less as shown in Fig. 23.

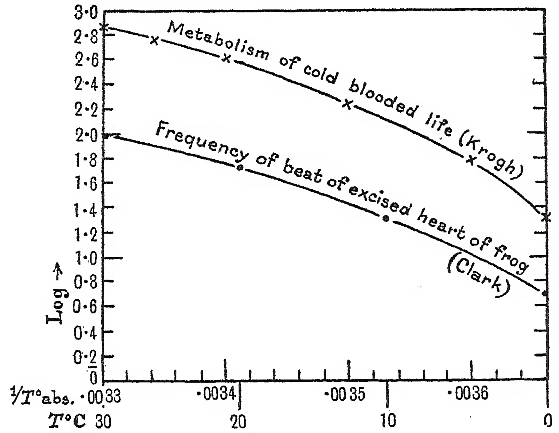

Fig. 22.

It is clear that if we can postulate two such reactions, one of which controls the velocity of the phenomenon observed at one temperature, and another at a higher temperature, we can postulate more than two, in which case a curve of the type shown in Fig. 24 would be obtained.

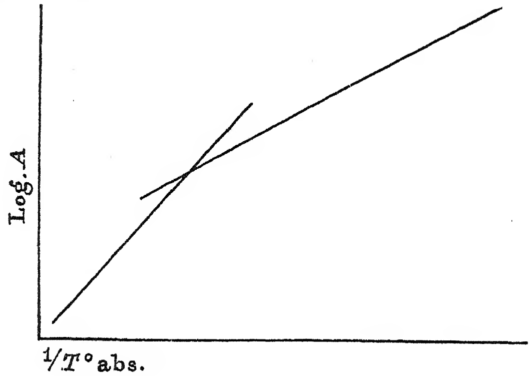

Fig. 23.

The numerous phenomena observed cannot, however, all be represented by a series of lines which cross one another after the fashion depicted in Figs. 23 and 24, and the observations fall rather into another scheme, that of a series of parallel straight lines as in Fig. 25.

It is not my purpose here to put forward arguments for or against Crozier's conceptions. Some of the criticism to which they can be subjected is of a very obvious character—some might think it so obvious as to be "cheap." For the rest the reader may be referred to an article in *Biological Reviews* by Bělehrádek (1930).

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For my own part I can only say that I started with a prejudice against the scheme which I have sketched out; but having tried to do some experiments with a semblance of accuracy and without any thought of the patterns into which the plotted observations would fall, and having found the points to drop with curious fatality into one or other of Crozier's schemes, the prejudice has passed away.

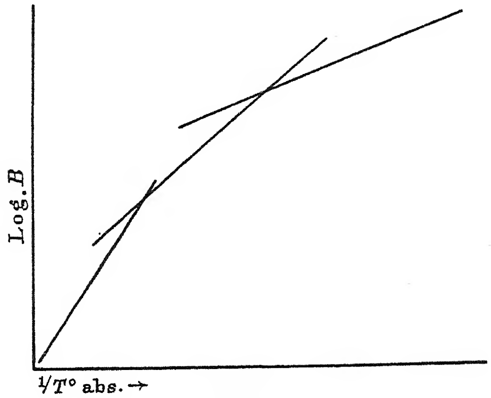


Fig. 24.

It is not my purpose here, as I say, either to support or to repudiate Crozier's point of view. It is my purpose, however, to say this: whether you accept Crozier's standpoint or another, it is clear that nature has learned so to exploit the biochemical situation so as to escape from the tyranny of a simple application of the Arrhenius equation. She can manipulate living processes in such a way as to rule, and not to be ruled by, the obvious chemical situation. That is true at least over a wide range of temperature.

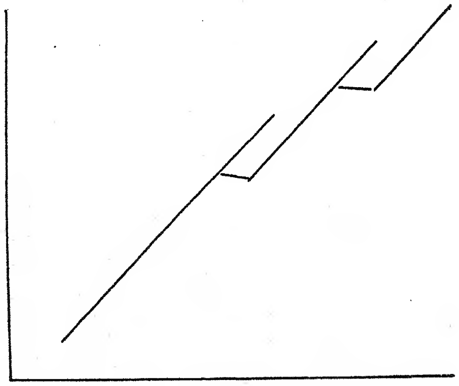


Fig. 25.

Having said so much let us pass to the consideration of certain individual phenomena in relation to temperature.

ENZYMES.

I used the phrase "living process" a few lines back: possibly that was not a very happy phrase. Whether any individual single chemical process in the body can be described as "living" is far beyond the scope of my present argument, but among the types of chemical action which are most closely associated with life none is more prominent than that of enzymes.

Therefore it is fitting that I should commence what I have to say with a reference to the effect of temperature on enzyme action.

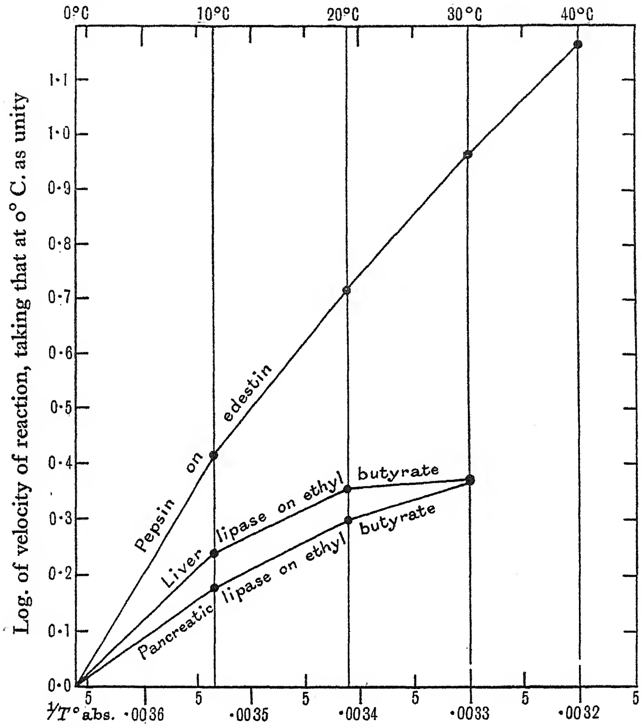


Fig. 26.

Different actions have very different temperature coefficients, but for the most part the value decreases as the temperature rises. The degree of decrease varies greatly in different cases.

I have plotted some examples which are at once typical and well attested, selected from those given by J. B. S. Haldane (1930).

The point I wish to emphasise in the present connection is that lipase in some way or other has become nearly independent of temperature at the highest temperature at which it has been studied, this being already considerably below that of the homoiothermic animals. How has this occurred? We do not know; yet it seems to be a most interesting and striking case of adaptation by what I have called the method of evasion. Clearly it is not possible to say that the temperature is "buffered," for the actual alteration in temperature takes place. The result, however, is much the same; the chemistry of the animal is so twisted that the alteration in temperature imposed upon it has but little effect on the velocity of the reaction. The objection has been raised to some of the above examples, that the medium becomes more acid as the reaction proceeds. This objection may from one point of view be valid; from another it may provide a hint as to how such things can happen in life.

Lipase is not the only enzyme which over a considerable range has rendered itself independent of temperature, as is shown by R. P. Cook (1930). The author gives three instances, namely the oxidation of $M/150$ formate (see Fig. 27), $M/60$ lactate (see Fig. 28) and $M/60$ succinate by toluene-treated *Bacillus coli* (incidentally we are far enough down in the scale of life) in each case; the oxidation is conceived as a process depending upon the abstraction of hydrogen in a molecule activated by a "dehydrogenase" in the bacillus and the transference of hydrogen to a "hydrogen acceptor," in this case oxygen or methylene blue. One can easily imagine that from the point of view of the bacilli as independent organisms, it is undesirable that these should be limited by the alterations of temperature of their hosts.

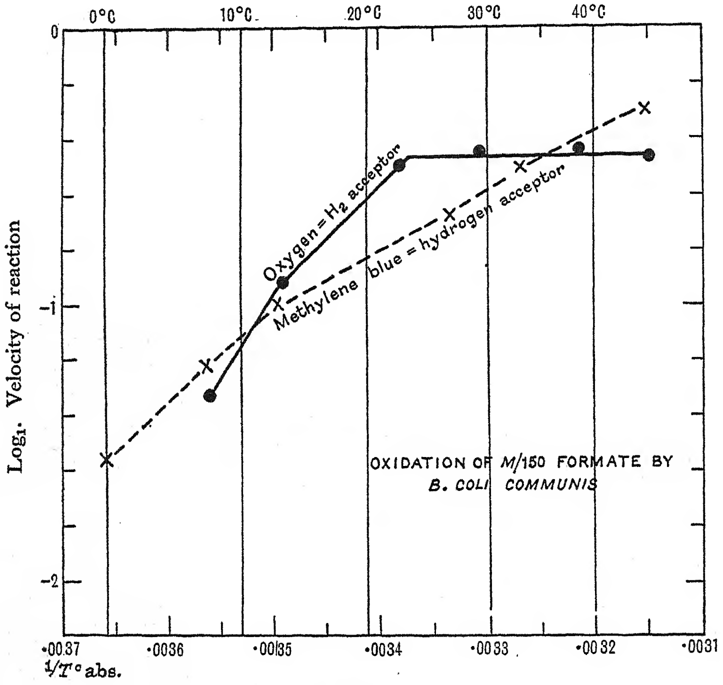


Fig. 27.

When methylene blue has been used as the acceptor the value of the temperature coefficient falls off rather gradually as the temperature rises, but when oxygen is used, the temperature coefficient becomes almost unity, *i.e.* the rate of the enzyme action becomes almost independent of temperature. It is to me extremely interesting that the action in question should be an oxidation, because there are very few chemical actions known which have a temperature coefficient of unity. Of these the reaction which has been most closely studied is also an oxidation and is one fundamental to many forms of life, the union between haemoglobin and oxygen as investigated by Hartridge and Roughton (1925). When I asked Roughton what explanation he could give of so extraordinary a phenomenon he said: "The only one I can think of is that over the range of temperature involved every molecule of oxygen which impinges on the haemoglobin sticks."

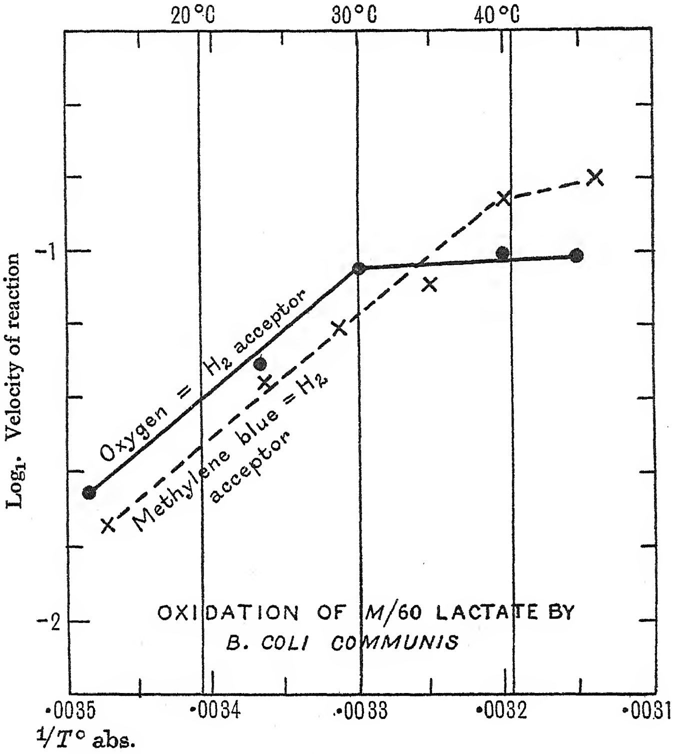


Fig. 28.

On the other hand, in the cases which involve enzyme action there is probably a much more complex phenomenon than in the reaction of oxygen and haemoglobin. As was pointed out to me by Brinkman, a combination of a chemical action with a positive temperature coefficient linked to an adsorptive one with a negative temperature coefficient might produce the type of result which is shown in Figs. 27 and 28.

OXIDATION OF YEAST.

We pass from the rather indefinite lowering of the temperature coefficient with rise of temperature, shown by some enzymes, to something which appears much more definite, namely the usage of oxygen by yeast cells.

Four experiments, each carried out with meticulous accuracy by Stier (1932), gave results which tallied perfectly with the type shown in Fig. 23.

The oxidation of yeast may not be at all simple, yet it seems likely to be among the simplest of living phenomena. The results are clear cut. It is certain that the temperature coefficient is lower at the higher temperatures, and it is much more difficult to fit any smooth curve to the points than it is to fit two or perhaps three intersecting straight lines.

THE HEART BEAT.

With the object of ascertaining something about the factors which were involved in the regulation of the heart beat in relation to temperature, Izquierdo (1931) and I carried out some experiments on the heart of the frog. The plan was to compare the effect of temperature on: (*a*) the rate of the perfused heart (which of course was necessarily free from humoral or nervous control from without), and (*b*) the rate of the heart in the intact body of the frog.

It happened that one batch of experiments was carried out in January, the other in June. In January on the excised heart the relation between the logarithm of the pulse rate and the reciprocal of the absolute temperature was a linear one up to 20° C. or thereabouts, beyond which the heart ceased to function. The machine jammed. That at least was the usual but not the invariable finding. Such a case is illustrated in Fig. 29. In a minority of cases the pulse rate fell off relatively to that demanded by the Arrhenius equation, as the temperature rose. It is interesting to note that out of such cases it was possible to pick instances which when plotted conformed to one or other of Crozier's patterns. Such a case is found in Fig. 30. There can be no doubt in this case of the sudden jump in the line at 15–17° C. But need this break always be sudden? Such instances as shown in Fig. 31 could easily be explained on the assumption that the displacement of the upper part of the curve was not sudden but gradual (Fig. 32).

The fact that we have obtained all Crozier's patterns from the beat of the frog's excised heart seems to indicate that the difference between one pattern and another is not fundamental, and it is clear that all the patterns could be obtained on the supposition that the cause of the break in Fig. 30 need not necessarily be so sudden as shown in that figure. I speak in this field with great diffidence, because I have only pottered about its edge. Crozier and his school have developed the interior with extraordinary care and beautiful experimentation.

When the excised heart of the frog was studied in summer, the frequency of the sinus beat gradually fell further and further short of that demanded by the logarithmic relation as the temperature rose, and indeed approximated to a new relation, namely a simpler arithmetical proportion between the temperature and

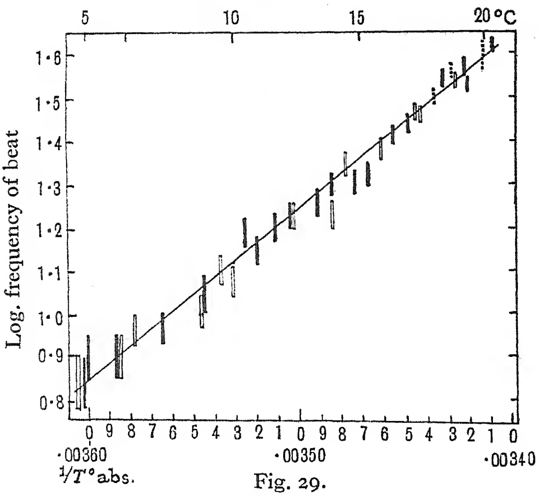


Fig. 29.

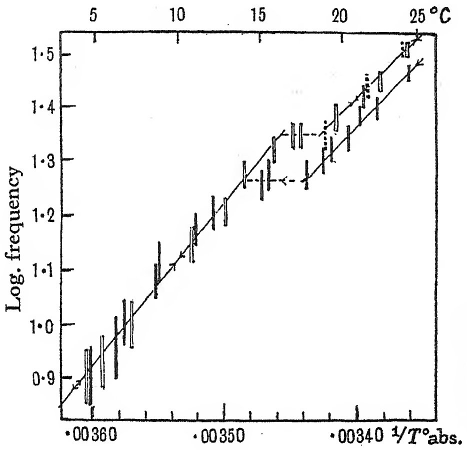


Fig. 30.

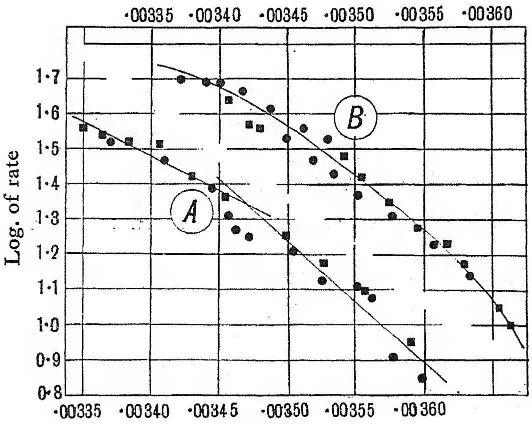


Fig. 31.

the frequency (Fig. 33). In the case of the frog in summer there was some gradual influence which became more and more prominent as the temperature rose, which influence increasingly depressed the velocity of the pulse. What this influence was we do not know. The simplest assumption no doubt would be that the factors which in the winter time operated in spasms at certain given temperatures, in summer came into the field more gradually, thus smoothing off the curves. One day Crozier will tell us whether or no this is so—my immediate point is another, namely to emphasise the fact that this departure from the law of Arrhenius is shown in the excised heart and not merely in the intact animal. It was in the intact animal that we first observed it, and it would have seemed reasonable to suppose that the

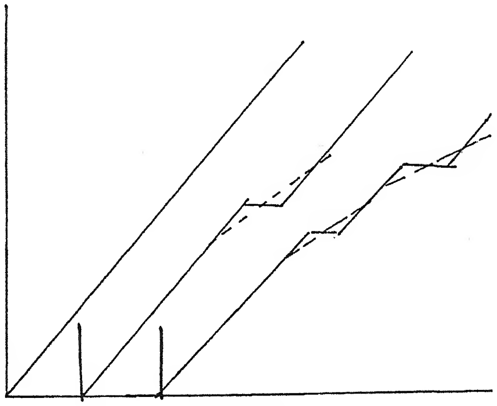


Fig. 32.

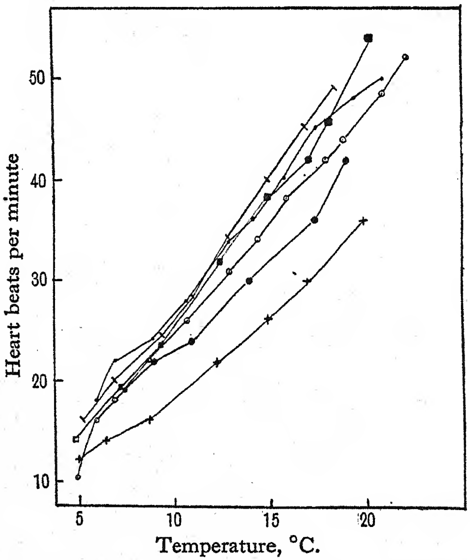


Fig. 33. Common frog, excised heart. Summer.

damping of the heart rate with temperature was connected with the very intactness of the organism, that, for instance, it was due to vagus impulses coming into the field or to the secretion of some hormone. Such an explanation is ruled out by the fact that the curves which relate the temperature and the heart frequency are practically identical whether the object of study be (1) the isolated heart, (2) that of the normal intact frog, and (3) that of the atropinised intact frog (Fig. 34).

The range of temperature over which the heart of the common frog beats (about 20° C.) is all too small to bring out the distinction between the logarithmic and the linear relation between temperature and frequency, another 10° would make a great difference. In this respect we had a stroke of good fortune. Prof. Hogben, then stationed in Cape Town, came into the laboratory and, sympathetic as ever, he pointed out that the South African clawed toad (*Xenopus laevis*) could withstand change of temperature of something approaching 40° C. Not only so but he very kindly sent in a consignment of these toads.

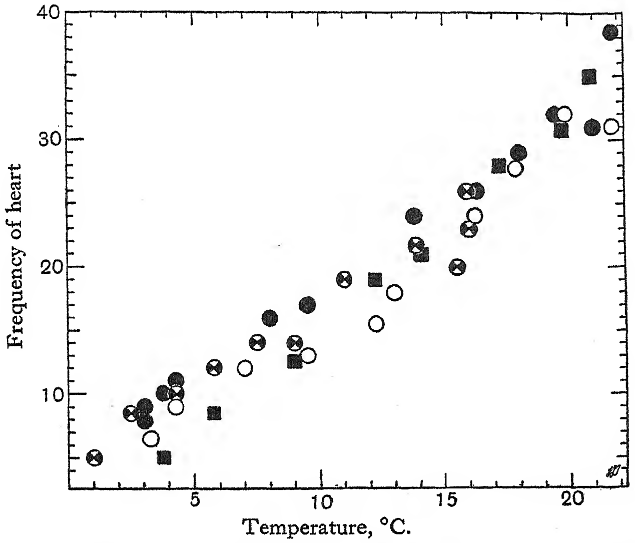


Fig. 34. Common frog, heart in intact animal. Summer: ⊗ going down, ○ going up. Atropinised: ● going down, ■ going up.

Fig. 35 shows the comparison (made by N. B. Taylor, 1931) between the excised heart of the common frog (*Rana temporaria*) and that of *Xenopus*. Over the range covered by the former, the curves given by the two species agree in type and in fact almost coincide; the curve from *Xenopus*, however, continues in its linear direction up to almost 30° C., then it falls off, so that at 33° C. the rate is less than at 29° C. If the temperature be further raised there is an abrupt increase in the sinus rate followed by death. The last phase must be regarded as beyond the region of function because it is irreversible. It appears to be due to a circus movement, and once it has supervened death is in any case only a matter of a short time.

In what may be regarded in the excised heart as "physiological," *i.e.* between

1° C. and 30° C. or thereabouts (differing by a degree or two in different hearts), the pulse rate bears a roughly linear relation to the temperature and not a logarithmic relation.

And now to pass to the heart in the intact animal. There was this great difference between *Xenopus* and *Rana*, namely, that the vagus exerts a marked action in *Xenopus*. This action appears to be almost absent at low temperatures, at its maximum at moderate temperatures, and it falls off at high ones (Fig. 36).

As compared with the excised heart, that in the intact animal shows another departure, the rapid rise of rate before death has never been obtained.

Fig. 37 shows schematically the relation between the curves obtained from the excised (*a*) and intact heart (*c*) and the reasons to which the differences between the two may be attributed.

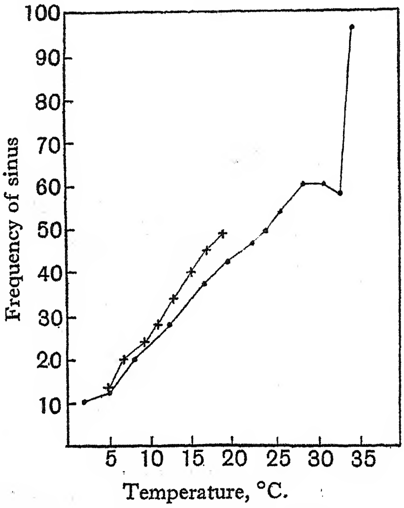


Fig. 35. Comparison of typical temperature-excised heart rate curves of British frog and South African toad respectively. + British; • South African. (N. B. Taylor.)

This in fact is the type of curve given by the heart of the intact *Xenopus*. The beats were counted by the string galvanometer, and the temperature measured in the bowel.

Yet the matter cannot be disposed of quite so simply. If so the atropinised intact *Xenopus* heart should give the same curve or nearly the same as the excised heart. That was so with the heart of the common frog. In the few experiments which have been carried out on *Xenopus* the atropinised heart gives a curve, intermediate between that of the normal heart and of the excised heart. The suggestion of two summits remains.

The natural transition from the lower vertebrates to the mammalia is by way of hibernating mammals. In the present connection the marmot has been, of these, the most completely studied.

The heart of the marmot will beat through the same range of temperature as has been discussed for the frog; the result for the perfused marmot's heart (as found by Endres, Matthews, Taylor and Dale, 1930), however, differs from that obtained for the perfused frog's heart. If the perfusion was started at 28° C. and the temperature gradually lowered, the heart beat, normal in character, became slower until about 17° C. was reached. Over that range the rate of beat had conformed to the logarithmic law and showed a temperature coefficient of almost exactly 2. Between 17° C. and 16° C. the heart beat suddenly became much slower (see Figs. 38 and 39). The reason was at once revealed by the string galvanometer record, the P wave had disappeared from its normal position and the ventricle was beating with a new and slower pacemaker.

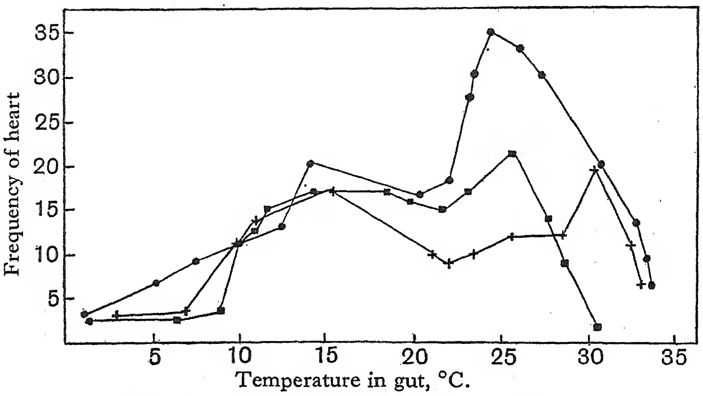


Fig. 36. Curves obtained from experiments upon intact heart of South African toad. Temperatures recorded by means of thermocouple in the bowel. (N. B. Taylor.)

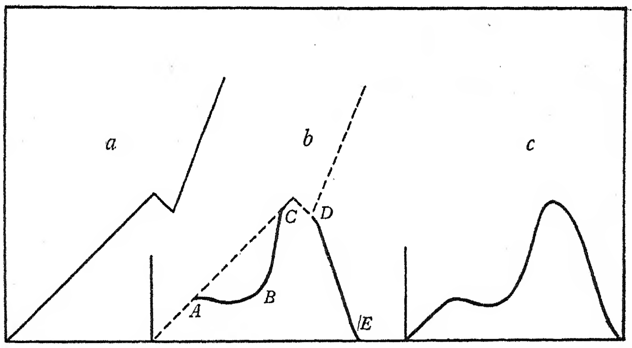


Fig. 37. A, B, C = vagus effect. D, E = period of death without circus movement.

In the marmot—again differing from the common frog—the reaction to temperature of the heart of the intact animal differed greatly from the reactions of the perfused heart.

In the short series of experiments performed in Cambridge lately, it early became apparent that special arrangements were required for the registration of temperature; it was therefore recorded both in the heart itself and in the rectum. At present we are concerned only with the temperature of the heart itself. The experiment now to be described commenced with a heart temperature of about 10° C. At this temperature the heart was irregular, about six beats per minute on the average, and the electrocardiogram did not indicate a definite *P* wave. As the temperature gradually rose the heart steadied, and at 13° C. the heart was beating regularly and with a normal electrocardiogram. At about 14° C., however, the whole picture altered, and by 17° C. the heart beat rose to 100. It was clear that something catastrophic had taken place (Fig. 40). The marmot was awakening.

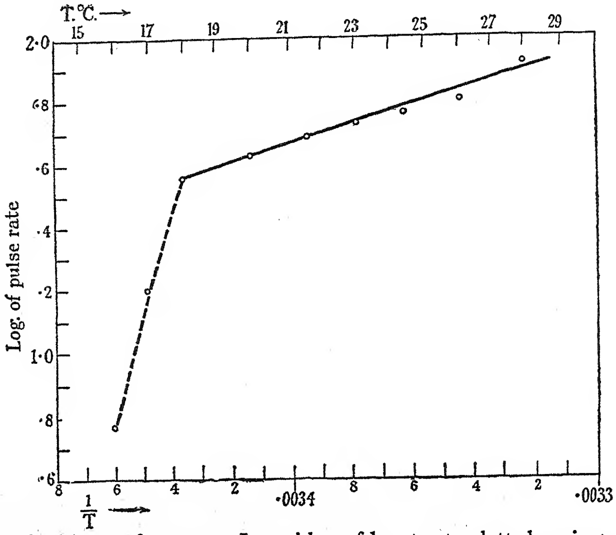


Fig. 38. Excised heart of marmot. Logarithm of heart-rate plotted against reciprocal of absolute temperature. (By permission of the Royal Society.)

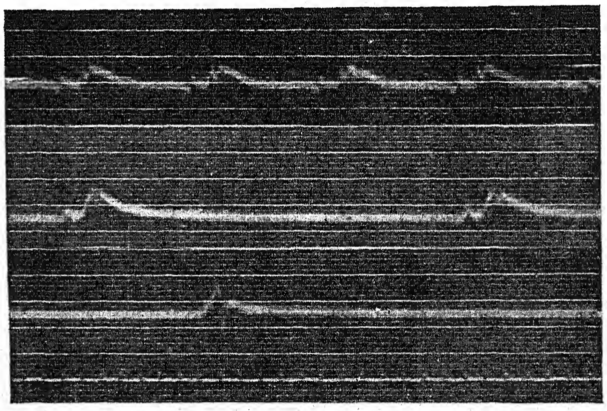


Fig. 39. Electrocardiogram of excised and perfused heart of marmot at 25·2°, 17° and 16° C. respectively. (By permission of the Royal Society.)

There is no real analogy between the sudden rise shown in Fig. 40 and that shown in Fig. 38, because electrocardiograms taken at temperatures of 10–12° C.

show well-marked *P* waves (Fig. 41). Indeed it might be contended with some show of reason, though I think it could not be said with certainty, that the *P* wave persists at a lower temperature than do the ventricular waves (Fig. 41 *c*).

No; a more probable explanation of the sudden rise shown in Fig. 40 is along lines suggested by the work of Gellhorn (1924). Gellhorn shows that adrenalin increases the temperature coefficient of the frequency of the heart. Clearly an outpouring of adrenalin would at once produce the sort of sudden rise which occurs when the marmot awakes and would also account for the higher value for the temperature coefficient in region 16–21° C. as compared with that at 11–14° C.

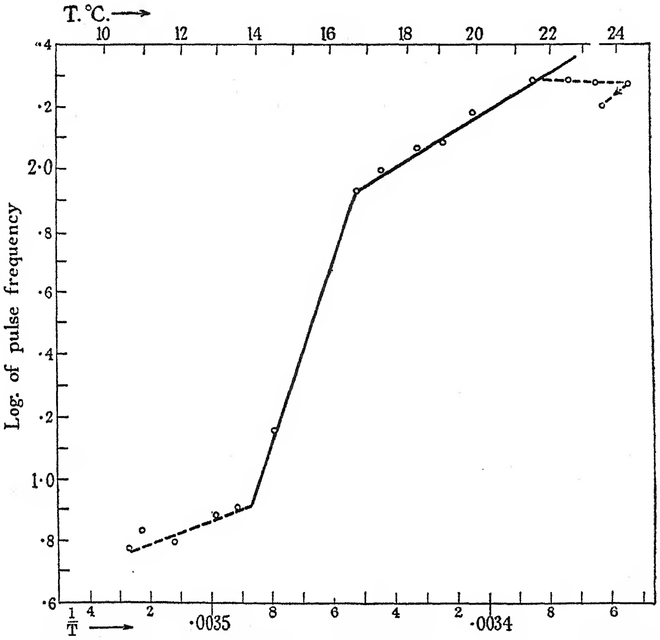


Fig. 40. Heart of intact marmot. Relation of log. of frequency to reciprocal of absolute temperature. (Upper scale = temperature in °C.) (By permission of the Royal Society.)

Such a pouring out of adrenalin is not merely a manifestation of the activity of the suprarenal medulla, behind that are stimuli arriving along the splanchnics and behind them is the brain.

Evidently the whole venue had changed; no longer were we studying the effect of the temperature on the heart beat as such, but the effect of temperature on the brain, and here at the threshold of homoiothermism we encounter the sympathetic system as a factor in the adaptation of the heart rate to temperature.

The awakening of the marmot seems primarily to involve quite a high level of the brain; the mere difference between wakefulness and sleep according to Hess (1929) is a manifestation of altered activity of certain centres in the brain, whilst the most obvious signs of the awakening in the marmot are intense shivering and the invocation of the whole mechanism for the augmentation of heat production. In this mechanism acceleration of the heart is merely an incident.

Attention has been drawn to the increase in the value of the temperature coefficient in the region around 20° C. If the temperature coefficient in that region were maintained the pulse would soon be racing, but another factor enters, namely, the vagus. At 23° C. (Fig. 42*a*) the pulse rate is uniform, at 26° C. (Fig. 42*b*) signs of arrhythmia are seen, whilst in Fig. 43, at 29° C., the arrhythmia is of the most pronounced type.

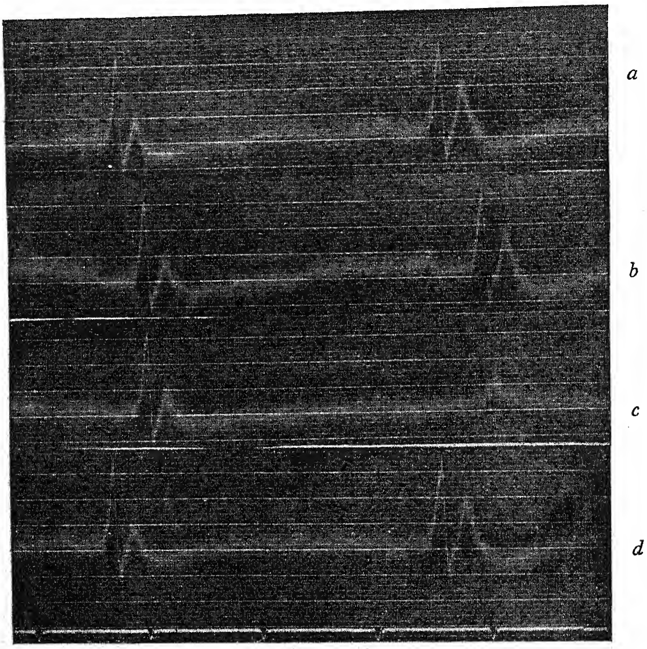


Fig. 41, *a–d*. Four consecutive pairs of heart beats, which take place at quarter-minute intervals. Time marker, 1 second. (By permission of the Royal Society.)

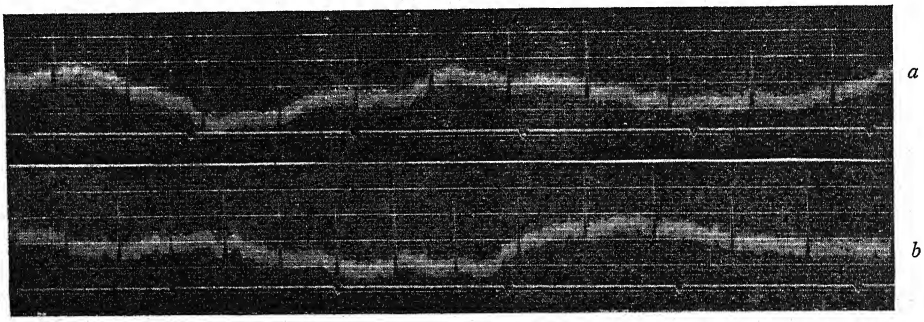


Fig. 42, *a* and *b*. Electrocardiograms at 23° C. and 26° C. respectively; the former shows no arrhythmia, the latter shows commencing arrhythmia. Time marker, 1 second. (By permission of the Royal Society.)

The contrast between the effects of temperature on the heart, when the heart is and is not harnessed to the thermogenic centre (if I may use the term) is well shown in the cat. As is well known, animals when deeply narcotised lose their power of heat regulation and become in effect poikilothermic.

If the deeply narcotised (luminal or chloralose) cat be cooled the rate of heart beat falls progressively with the fall of temperature and the cat remains quiescent. It behaves much as the frog would do under similar circumstances. The velocity of the pulse always varies in the same sense as the temperature of the cat. Indeed, like the heart beat of the frog and like the excised mammalian heart (Knowlton and Starling, 1912), the pulse is, from 33°–44° C., roughly a linear function of the temperature (Fig. 44*a* and *b*) (Barcroft and Izquierdo, 1931*a*).

If on the other hand the cat be narcotised very lightly (no more than sufficient to make the handling of the animal possible), quite a different picture presents itself. The pulse, as the animal is cooled below the normal body temperature, tends to rise instead of fall, and that tendency to rise is maintained over a drop in temperature of several degrees.

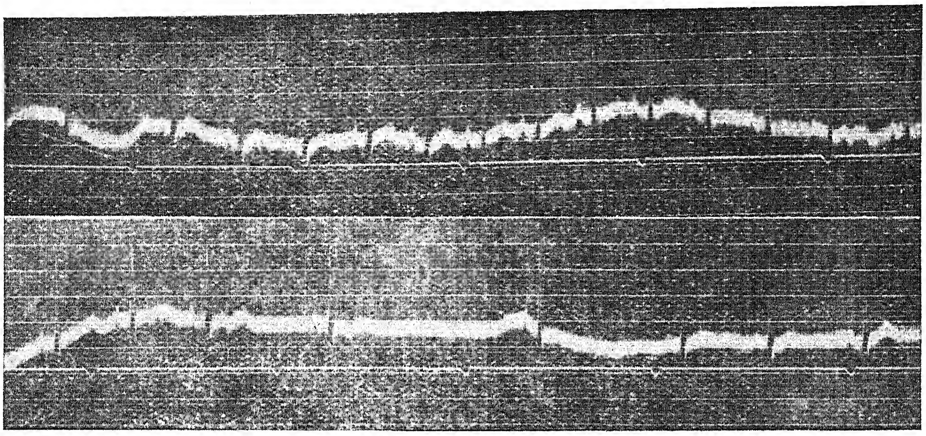


Fig. 43. The upper and lower portions read continuously. Same experiment as Fig. 42, 29·10° C. Arrhythmia well established. Time marker, 1 second. (By permission of the Royal Society.)

Fig. 44*b* shows the mean pulse rate, but in point of fact it is probably not fair to speak of a mean pulse rate in the region immediately below the normal body temperature. The fact is that the pulse rate fluctuates from moment to moment in a quite unusual and abnormal way. The line of maximum pulse rate represents a summit, the line of minimum pulse rate at any temperature seems to be the smooth curve such as would have been given by the deeply anaesthetised animal. A new phenomenon then faces us, that of a mechanism invoked by cold which accelerates the pulse, but it is a mechanism independent of vagus activity, for similar tracings may be obtained whether the cat be atropinised or not.

It is clear that the acceleration of the heart beat on cooling is, again, purely incidental, the cooling is associated with shivering, biting and the general symptoms of sham rage which have been made familiar to us by Cannon and Britton (1925), Bard (1928, 1929), and other members of the Harvard School, and once more we are studying the effect of cold not on the heart but on the base of the brain. The efficiency of the heart itself is simply taken for granted.

Clearly more striking results might be expected could the experiments have been carried out on the completely unanaesthetised animal. The unanaesthetised cat does not lend itself to such experiments. If man be substituted, there is much to be gained. The anaesthetic may be dispensed with, he is higher up the animal scale, and there is the additional interest of any subjective observations which may be obtained.

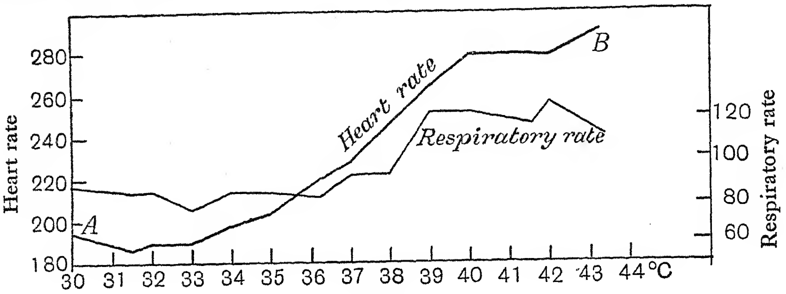


Fig. 44*a*.

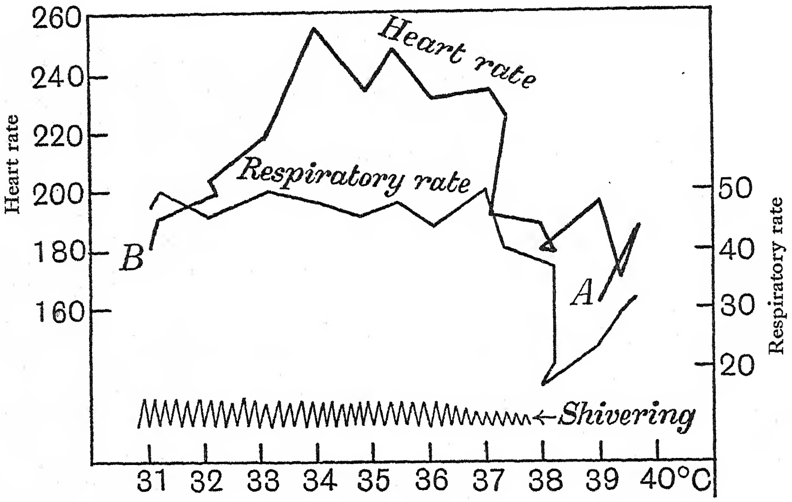


Fig. 44*b*.

Fig. 44*a*, from deeply anaesthetised, and 44*b* from lightly anaesthetised cat, ordinate heart and respiration rates, abscissa temperature °C.

Four experiments have been carried out on man, the general scheme of which was as follows. The subject lay naked, in bed, in a room at 3–4° C., amply protected from the cold by bedclothes until his pulse had become steady. The bedclothes were then removed with the result that he commenced to shiver. The shivering soon became violent and was accompanied by marked dyspnoea. Each gasp consisted of several inspirations imposed upon one another without and unseparated by commensurate expirations. The heart rate showed the same sort of irregularities as that of the cat but on a much more striking scale (Fig. 45), for the whole drop in rectal temperature over about half an hour was less than 1° C.

When the bedclothes were replaced and without any rise in body temperature the shivering passed off and the pulse dropped to a level below that observed after it became stabilised at the commencement of the experiment. The experiments on man yielded in a more definite form what those on the cat had rather indicated, that the rise of pulse rate which takes place on exposure was part of the same syndrome as the shivering. Moreover, confirming the results of Magnus and Liljestrand (1922), it appeared that the shivering was itself due to a reflex from the skin, for as soon as the skin was warmed the shivering ceased.

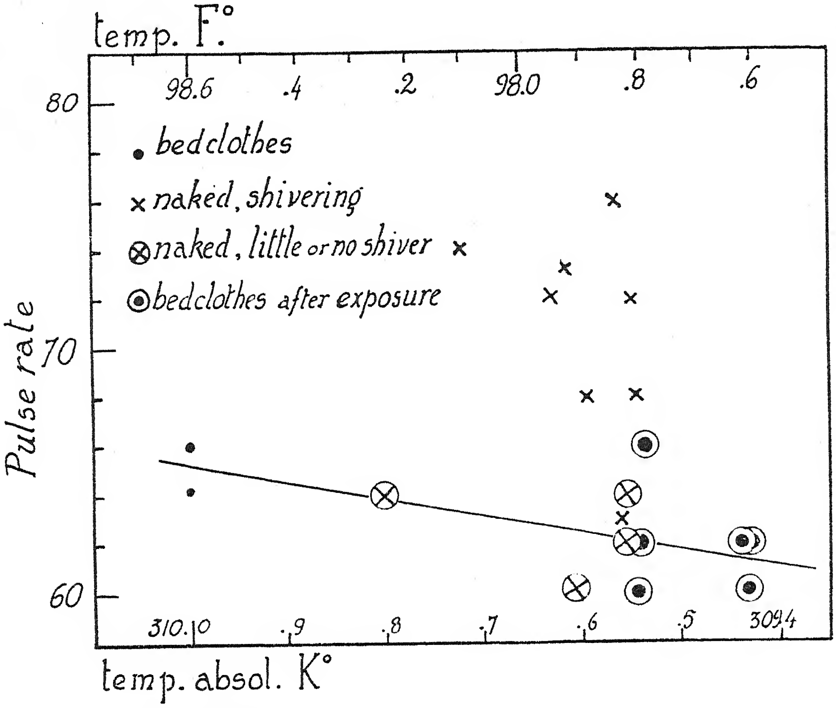


Fig. 45. Relation of rectal temperature to pulse rate in man.

It seems possible to couple these experiments with those of Cannon (1925), who induced shivering and a rapid pulse rate in cats by placing ice-cold water in the stomach, or by placing the cats in a draught. Unfortunately the body temperature is not recorded in these experiments, but the point which appeared was that the results depended upon the intactness of the sympathetic system, for they were not obtained in animals deprived of it.

To sum up, therefore, what we have said about the effect of temperature on the heart is this: That in the frog in winter the excised heart usually obeys the logarithmic law relating its frequency to the temperature; in exceptional cases curves may be obtained showing Crozier's patterns; that in summer it is subjected to temperatures so high that the logarithmic relation would enforce much too high a heart rate; that by some process the frequency has been damped at higher temperatures, making the relation between frequency and temperature nearly linear; that the damping process is to be seen in both the excised and the "intact" heart and has nothing to do with vagus or sympathetic action. In the South African toad, which has a much larger range of temperature, the basal arrangement appears to be quite similar to that of the common frog (*Rana temporaria*) in summer, but in addition the vagus comes into the field and slows the heart even more than otherwise would have been the case.

The heart of the hibernating marmot when excised and perfused follows the logarithmic law between certain temperatures. Above about 30° C. it "jams," below about 15° C. the heart becomes very slow, a nodal beat only taking place. In the living animal the heart has a range of temperature from about 1° C. to about 30° C. The relation of the logarithm of the frequency to $1/T$ is linear, but with a great break at about 15° C., where the heart suddenly accelerates (at higher temperatures acceleration is much less and there is very pronounced vagus action).

In the deeply narcotised cat there is a rise of pulse rate with rise of temperature, but if the narcosis be light the fall of temperature for some degrees below the normal body temperature produces a rise in pulse rate, which apparently is associated with shivering and depends on a nervous factor superposed upon and overruling the more fundamental relation seen in cold-blooded animals.

Here then is a very fine issue—the cold-blooded animal successfully adopting ingenious mechanisms, first biochemical, then physiological, in order to adapt its heart to the variations of its environment; the warm-blooded animal discarding what its cold-blooded predecessor has laboriously beaten out, invoking the nervous system to reverse the normal biochemical relationship and gaining a new freedom by adapting, not itself to the internal environment, but the internal environment to itself.

RESPIRATION.

Our consideration of the effect of temperature on the heart beat has quite failed to show that a constant temperature is of any advantage to the heart; on the other hand, that organ can function quite satisfactorily over a great range of temperature. Moreover, at the point at which temperature regulation effects a homoiothermic state the heart becomes a mere servant, its own interests being apparently taken for granted.

While respiration is naturally associated in the mind with the thorax and even with the heart it is constantly necessary to recollect that essentially respiration is conducted in the brain. Our study of the effect of temperature on respiration is the study of the reaction of cephalic—not thoracic—processes to environment. That indeed adds additional interest. For while it might reasonably be held that the organs of the body had, before the evolution of the thermotaxic mechanism, been so evolved as to be efficient over a great range of temperatures, it might equally be held that once the brain is touched constancy of environment is all important, and that "la vie libre" involves the whole of the efficiency of cephalic processes.

Yet the study of the effect of temperature on respiration leads us into a room in which the pictures are curiously similar to those which we have just left.

We may start as low down the animal kingdom as we please. Crozier and Stier (1925) have determined the temperature coefficients of the respiratory movements of various forms of insect life, and have applied their methods to the observations of other workers.

Here I need only refer to two sets of readings, one on the larva of *Anax* which gives a straight line when the logarithm of the frequency is plotted against the reciprocal of the absolute temperature, the second being on a decapitated grasshopper. This latter shows one of the characteristic patterns which we have already seen in heart tracings. But in each case the respiration is capable of accommodating itself to temperatures which cover a considerable range, in the case of the grasshopper 18° C.–40° C. Our interest, however, is more directly with the vertebrates, and therefore we may turn to the respiratory rhythm of the goldfish, which is of special interest in view of the observations of Adrian and Buytendijk (1931), to which allusion has been made in a previous section. Many workers have compared the frequency of the opercular movements with the alterations in the temperature of the water. Crozier and Stier (1925), who give a comprehensive account of the subject, show that over a range of about 20° C., from about 8–26° C., the frequency of the opercular movements obeys the logarithmic law and varies with temperature in precisely the same way as the oxygen consumption of the fish.

Passing from the goldfish to the frog, Crozier and Stier have plotted the relation between pharyngeal movements and the temperature, again plotting the logarithms of this frequency against the reciprocal of the absolute temperature. In preparations the forebrain of which is destroyed there is at least the suggestion of a break, at about 20° C. (reciprocal 0·00341), of exactly the same nature as the break which we have seen in the corresponding curve for the heart.

Before passing to the mammalia I should perhaps point out that while the gill movement of the fish may morphologically be the parent of the gasp, the pharyngeal movement of the frog occupies a rather ambiguous position. It is not admitted by Crozier and Stier that the pharyngeal movement is, as is generally supposed, the true respiratory movement. They regard the movement of the throat as serving merely to renew air within the buccal cavity while the glottis is closed, and as "not proper movements of lung ventilation (Baglioni, 1900, 1911), although presumably innervated from the respiratory centre (for review of innervation see Black, 1917). The flank movements, indicating lung filling, seem, however, to follow the same curve so far as our observation extended."

In the deeply narcotised cat (Barcroft and Izquierdo, 1931*a*) the rate of respiration bears a rough logarithmic relation to the reciprocal of the absolute temperature between the temperatures of 31° C. and 41° C. and therefore bears the sort of relation which might be expected in a cold-blooded animal (see Fig. 46). Each respiration appears to be normal in kind, and it does not seem possible to say that the mechanism of respiration judged as such is at a disadvantage. But when the narcosis is very light the whole picture is altered, as it was in the case of the heart beat (see Fig. 47).

The cardiac rhythm, we saw, became an incident in the mechanism of heat production. The connection between the respiratory rhythm and the thermotaxic mechanism is even more intimate. Indeed, in animals which do not sweat respiration is not merely connected with the machinery for temperature regulation, it *becomes* in great part the machinery for temperature regulation. That is a beautiful inversion; the mechanism for the evasion turned upside down and turned into the mechanism for correction.

Cat, deep anaesthesia

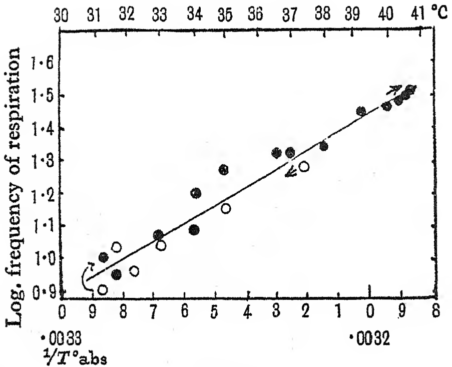


Fig. 46.

Cat, light anaesthesia

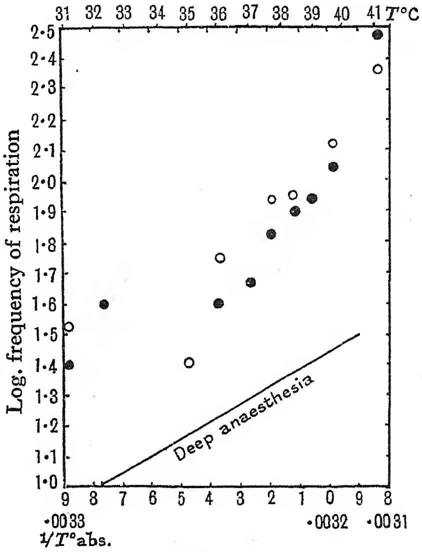


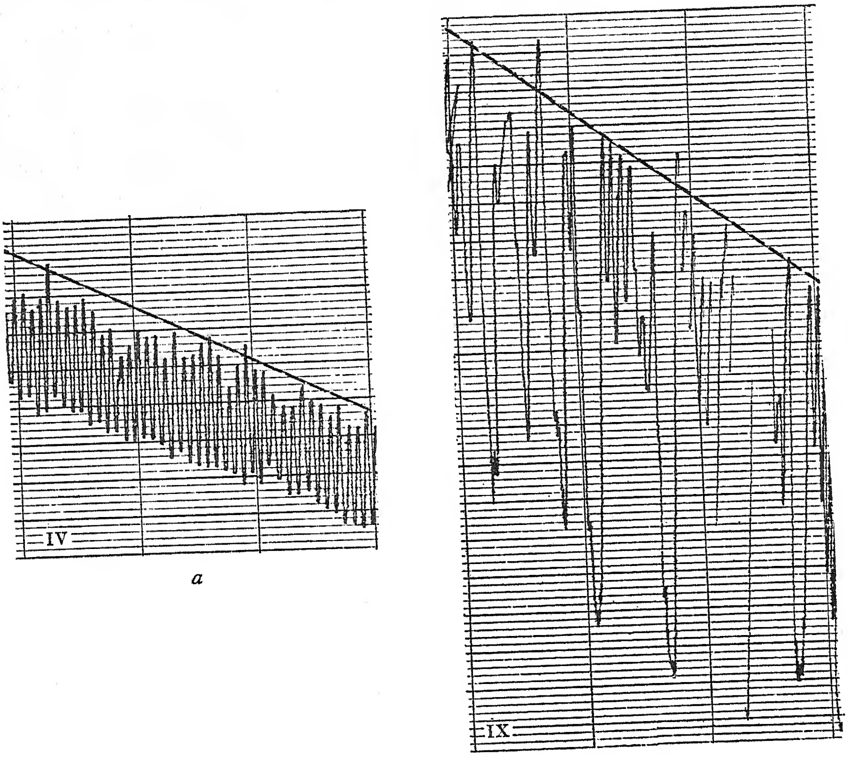
Fig. 47.

What then is the relation of respiration to heat regulation? This may be considered with regard to displacement of temperature on either side of the normal, first in relation to the lowering of temperature and secondly in relation to rise of temperature.

Relation of respiration to lowering of temperature in man.

In the experiments of which I have already spoken in which the human body was exposed to a low temperature, tracings were taken from which may be deduced both the ventilation of the lungs and the heat production of the subject.

Fig. 48 *a* shows the course of respiration at the normal temperature, and Fig. 48 *b* shows the same when the temperature was reduced to 36·6° C. The normal sequence of inspiration and expiration is abolished and is replaced by a series of very deep inspirations. On closer scrutiny it seems a little inaccurate to call them deep inspirations. The upstroke in each case is a series of inspiratory efforts, the expirations between them having disappeared. The upstroke is in fact just like that of a cyanide apneusis, and the breath is held for an appreciable time before the apneusis breaks down. We are dealing with a region of the brain at least as high as that which differentiates between pneumotaxis and apneusis. But that is not the whole story, because, as Dr W. G. Garrey pointed out in conversation beforehand, and observation confirmed him, the shivering fits with which respiration of this type is associated always occur on the inspiratory phase. The two are coupled together, suggesting that either inspiration causes shivering or shivering causes inspiration: of the two the former seems the more probable, namely that the activity of the inspiratory centre is allowed to irradiate in some way over the central connection of the muscles involved in shivering in much the same way as it irradiates over the centres which govern muscles of forced respiration to form a gasp. If it is not more than a slight exaggeration to say that a rigor is an apneusis run riot, it is not more than a slight exaggeration to say that the regulation of respiration becomes the regulation of heat production when the temperature is artificially lowered.



a

b

Fig. 48. Tracing of respiration taken with Dubois' spirometer: *a*, normal temperature; *b*, 36·6° C.

Relation between respiration and rise of temperature.

When the temperature is raised in man the principal vehicle of heat loss is the evaporation of sweat. In most of the higher animals this is not the case. In them heat loss is effected largely by the evaporation of water from the mouth and tongue and respiratory passages. The amount of heat lost depends upon the quantity of water evaporated, which in turn is a function of the volume of air which traverses the respiratory system. Nevertheless it is clear that ventilation of the lungs must be ruled by quite other considerations, namely the preservation of the constancy of CO_2 in the alveolar air, which in its turn depends upon the metabolism. The organism is therefore presented with the issue of how to reconcile two opposing elements: (1) in order to diminish heat production the metabolism should be reduced to the minimum with consequent reduction of the alveolar ventilation; (2) to promote heat loss there should be a maximum of evaporation with a consequent proportional increase in total ventilation. The body has found a solution in the phenomenon known as tachypnoea which you may see any day when a dog lies down in the warm sunshine—rapid respiration, but so shallow that the alveoli are little ventilated, whilst the amount of air which passes over the mucosa of the mouth, the windpipe and the bronchi is very great. The opposite combination of events occurs when the body is cooled, namely an increase of the metabolism and so of the CO_2 elimination whilst reducing the total ventilation to the minimum compatible with the preservation of a constant alveolar CO_2. The breath is then held for a while and exhaled. The total ventilation is increased, as it is in tachypnoea, but note that the process is of precisely the opposite type as regards evaporation. As the result of these large respiratory spasms the ratio of alveolar ventilation to dead-space ventilation is at its maximum, not at its minimum, and therefore there is the least degree of cooling for a given oxygen uptake. And so we are faced in most mammals (Equidae excepted) with a co-ordinated machine by which a mechanism evolved for the purpose of oxygenating the blood is pressed into service for the regulation both of hydrogen ions and temperature, and is so manipulated as to do the work whether these variables alter in the same or in opposite senses. Judged as a *tour de force* this mechanism must be almost without a rival, but it is not surprising that in man, the form of mammal which demands the finest adjustment both of temperature and of hydrogen ions, such a triple purpose mechanism, however ingenious, should be discarded and an installation provided for the independent regulation of temperature. This installation is the highly developed functional skin with its deep layer of material which is very retentive of heat when the vessels are constricted and its power of copious secretion designed to "eliminate" calories on the great scale.

The last point to stress is one which will not have escaped the reader, namely that the actual fight for the preservation of a constant internal medium is carried out in the brain—and if Barbour and Prince (1914) are correct, rather high up in the brain, namely in the corpus striatum. The mere locality in which its seat of exact temperature regulation is situated suggests that this is a development of the higher vertebrate types.

Our review in this section started with the enzymes which were on the borderland of life, we saw in them a mechanism which was of the nature of buffering, an acceptance of temperature variation and an adaptation of the organism to it, the method of "evasion," and we have ended by the reversion of the process, the refusal to accept alteration of temperature, and the domination of the method of elimination.

We started with a purely chemical phenomenon in a unicellular organism, we end somewhere just below the cerebral hemispheres.

IV. OXYGEN.

When Claude Bernard put forward the principle of constancy of internal environment he instanced three substances, one of which was oxygen.

The mention of oxygen raises a point which hitherto has not been discussed. The environment of the cell is not really blood but lymph, blood as an approximation merely. If the amount of any particular material in the blood is large, and if the amount lost during the passage of the blood through the capillary is small, the composition of the blood plasma may be taken as being a sufficiently close approximation to that of the lymph. In the case of oxygen, however, these conditions do not hold good. The blood loses a quarter to a third of its oxygen in passing along the capillary. What then is the closest approximation to the internal environment of this cell which can be expressed in terms of the composition of the plasma? Clearly the plasma of the venous rather than the arterial blood, for with it the lymph is most nearly in equilibrium.

At first sight it may seem that the oxygen content of the lymph would be more constant if it were governed by the oxygen in the arterial rather than the oxygen in the venous blood. The arterial blood is relatively constant in relation to its oxygen content, and the venous blood is relatively variable.

Points arise, however, to modify this conception: in the first place it is the concentration of oxygen in the *plasma* which counts. The haemoglobin is practically an oxygen buffer of a very complete kind and the oxygen dissociation curve may be regarded much in the light of an oxygen titration curve. By one of the miracles of nature it has the general properties of other curves which are obtained by the titration of buffered solutions, namely at the extreme ends of the curve a small addition of material buffered makes a large alteration in its potential, but towards the centre of the curve a large addition of the material buffered makes but a small alteration in potential.

At no point on the curve is the oxygen pressure (*i.e.* the concentration in the plasma) independent of the quantity of oxygen in the blood, but in ordinary oxygenated blood in the region situated symmetrically about that of half-saturation the blood can impart or take up over 40 per cent. of its possible oxygen content within an extreme range of pressure of 20 mm. Hg. But that is not all, for in the mammals carbonic acid exchange takes place approximately synchronously with oxygen exchange. As was shown by Christiansen, Douglas and Haldane (1914) the amount of oxygen which the blood uses for a given alteration of oxygen pressure is increased by the fact of its acquiring carbonic acid in nearly equivalent quantities. Therefore when we consider the dissociation curve of the arterial blood, and even more emphatically is it true of the circulating blood, very different degrees of unloading are consistent with very small differences in pressure. In Bock, for instance, the saturation of the venous blood varied between 77 per cent. and 41 per cent. On the curve for the circulating blood given by Christiansen, Douglas and Haldane, the difference of saturations would amount to about 15 mm. oxygen pressure. But here comes another point: when heavy exercise is taken such as would reduce the saturation of the venous blood to 40 per cent. the blood alters in reaction, the dissociated curve therefore shifts. This shift, as shown by the Monte Rosa Expedition of 1911, may result in as much as 7 mm. increase in oxygen pressure at 40 per cent. saturation. It is quite possible, therefore, that between such slight exercise as would admit of the venous blood being 75 per cent. saturated, and such heavy exercise as would reduce its saturation to 40 per cent., there is a pressure difference in the mixed venous blood of less than 10 mm. of mercury.

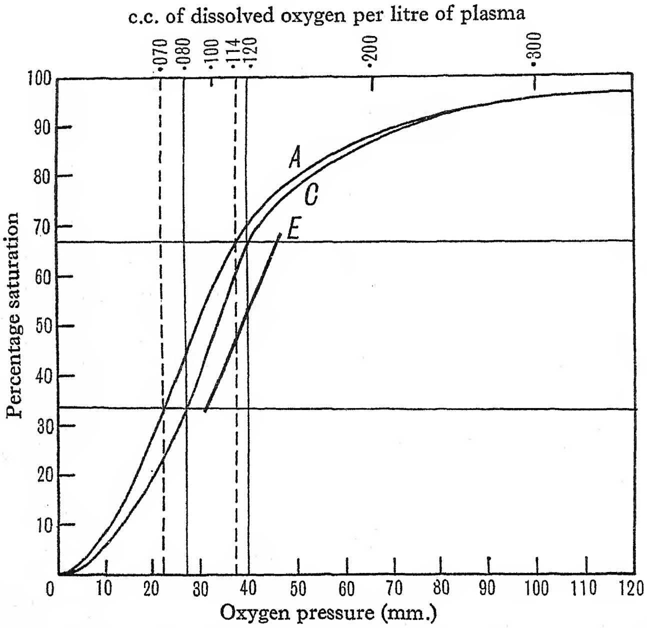


Fig. 49. A = arterial blood; C = circulating blood (rest); E = circulating blood (exercise).

To pass to the evolution of haemoglobin. It used to be said that haemoglobin had been evolved two or three times over. The statement had always seemed to me to be a little vague. I suppose it meant that haemoglobin had cropped up, apparently spontaneously, in several phyla of the animal kingdom which were not in the same line of descent. However that may be, the whole subject has taken on an entirely new aspect within the last six or seven years.

This progress has been due to the work of Keilin (1925, 1926), who originally was interested in the problem of whether the haemoglobin of the parasitic larva of *Gastrophilus* is the same as that of the host—namely the horse—in which it develops. *Gastrophilus*, which is a fly, and the horse are far enough removed phylogenetically, but is their haemoglobin the same, and if so is it merely the haemoglobin of the horse that is laid down in the tissues of the larva?

The two are spectroscopically different, but it might be that in any case the haemoglobin laid down in the tissue of an animal is different from that found in its blood, and that the more proper comparison would be between the haemoglobin in horse muscle and that in the larva of *Gastrophilus*. Hence an attack on the haemoglobin in muscle. The earlier part of Keilin's work was largely a confirmation of the work of MacMunn (1886, 1889). At the time of its publication it ran counter to the authority of Hoppe-Seyler's school (Levy (1889)), and on that account largely it did not come into its own. But so far as Keilin was concerned the muscle was a starting-point. Keilin found that a spectrum identical with, or almost identical with, that obtained from the reduced muscular tissue was to be observed over a great range of tissues. These tissues in the animal kingdom were spread over nearly every phylum: not only that, but the spectrum to which Keilin attached the name "cytochrome" could be seen in bacteria, yeast and plants.

What relation, if any, has cytochrome to haemoglobin? Anson and Mirsky (1925), about the same time, had definitely proved that haemochromogen was a conjugated protein consisting of haematin attached either to denatured globin or to one of a great many nitrogenous compounds, such as pyridine, nicotine, etc. These various haemochromogens gave spectra of the same type, but differing in detail. According to Keilin the rather complex spectrum of cytochrome consists of six absorption bands, three of which are so close together as apparently to fuse into a single band. This spectrum can be resolved into three separate spectra, each of two lines. Of the three two appear to be those of different haemochromogens. It is not my purpose here to discuss the third spectrum. The point is that over a great part of the animal and vegetable creations haematin is to be found in association with some nitrogenous compounds, on the same lines on which it unites with globin. Nor is this the whole story. Out of Keilin's research on cytochrome arose delicate methods of testing for haematin itself. The haemochromogen spectrum is seen in higher dilutions than that of any other blood pigments, and among haemochromogen spectra that of pyridine haemochromogen is pre-eminent for its visibility in low concentrations. Where haematin is suspected, therefore, it is only necessary to add pyridine in the presence of a reducing agent, and if haematin is present, even only in infinitesimal quantities, it will stand revealed.

Haematin has thus been discovered in many unexpected places, of which the growing points of certain vegetable tissues are not the least interesting. The interest lies largely in the fact that as the cell ages the haematin appears to be transformed into cytochrome, the haematin is therefore the more primitive material; and if the vegetable cell had contained the necessary globin, there is no reason why haemoglobin should not be found widely distributed in the vegetable kingdom. But to pass from the evolution of haemoglobin itself to that of its peculiar properties. The question naturally arises: Is the sigmoid oxygen dissociation curve which so resembles a titration curve in type an inherent property of haemoglobin, or is it something rather specialised?

The answer to this question has engaged the thoughts and energies of many interested in it for almost two decades, and still it is unanswered. Perhaps it never will be answered until we know the chemical nature of the linkage between haemoglobin and oxygen.

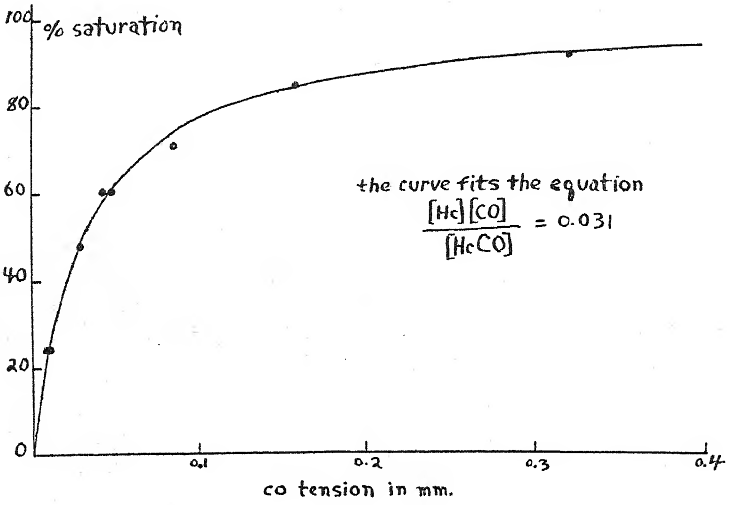


Fig. 50.

The following considerations, however, appear to be worth some thought.

(1) Haemochromogen does not yield a reversible compound with oxygen. The natural inference is that the nature of the protein (as between that of haemochromogen and that of haemoglobin) dominates the presence or absence of the essential linkages. Yet this view may be illusory, for it can scarcely be supposed that the nature of the haemoglobin-oxygen linkage is essentially different from that of the haemoglobin-CO linkage. Now CO unites reversibly with haemochromogen, and one may easily suppose that the reason why oxygen does not appear to do so is not because the essential linkage is lacking but because oxygen is a very active body in other respects, and that as soon as "oxyhaemochromogen" is formed some secondary transformation takes place (such as the oxidation of the iron) which destroys the system. If such a view is relevant, the equilibrium curve as between CO and haemochromogen at once becomes important, and that so far as we know is not sigmoid in character.

(2) In *Helix pomatia* there exists a form of haemocyanin in which the affinity of the pigment for oxygen is unaffected by the hydrogen-ion concentration of

the medium in which it is dissolved. The general parallelism between the properties of oxyhaemoglobin and oxyhaemocyanin is so close as to encourage the belief in the similarity of the oxygen linkage in the two compounds. There is no evidence of any sigmoid inflection of the dissociation curve of this oxyhaemocyanin in *Helix pomatia* (see Fig. 51).

Also, as I said earlier, Redfield has discovered in some worms a haemoglobin which possesses an affinity for oxygen which appears to be uninfluenced by hydrogen-ion concentration. The shape of its dissociation curve will be of great interest in this connection.

In the meantime one does not easily escape from the comment made by Dr and Mrs Stedman: "In view of the history of the oxygen dissociation curve of haemoglobin there will necessarily be some hesitation in accepting the implications of the results of the foregoing experiments" (1928). The experiments in question are those illustrated in Fig. 51. The allusion is of course to the fact that a dissociation curve for oxyhaemoglobin which is quite devoid of double inflection has never with certainty been produced. In 1914 we seemed to be "nearly there." It looked as though we had only to obtain haemoglobin a little purer than hitherto in order to obtain a specimen which yielded a hyperbolic dissociation curve. But further purification did not effect that result, and the fact remains that, unless it be the recent curve of Geiger (1931), no dissociation curve has been obtained from haemoglobin which does not present some suggestion of a double inflection.

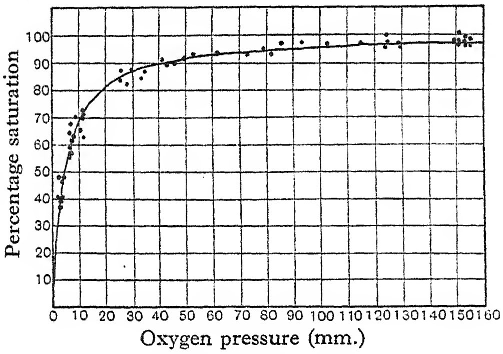


Fig. 51. Oxygen dissociation curve of blood of *Helix pomatia*, points range from pH4 to pH9.

So far we have considered haemoglobin as being simply a constituent of blood, but the amount of haemoglobin situated in muscle is in some animals quite considerable. It appears in respect of iron closely to resemble the haemoglobin of blood. In the lowest forms of life in which haemoglobin is found, its distribution is wider still: "In the annelid *Aphrodite aculeata* (Lankester) it is found in the nerve ganglia. The colour is most intense in the supraoesophageal ganglion which has as intense a colour as a drop of human blood....An exactly similar observation has been made by Hubrecht who found haemoglobin in the red coloured cerebral matter of certain worms which possess no coloured blood corpuscles" (Schäfer, 1898). What the function of haemoglobin in these lowly forms may be is quite obscure. Various workers have suggested that it holds a store of oxygen as against the times when the supply of that gas may be cut off.

In recent years the trend of opinion has been against this view, possibly because exaggerated claims were made for it. It always seemed to me suggestive, I cannot say more, that the lugworm, *Arenicola*, contained about as much haemoglobin in its body as could supply its consumption of oxygen from one tide till the next. Man carries in his blood less than five minutes' supply of oxygen, *Arenicola* five hours' supply—why?

In the mammals and birds, moreover, the only explanation which till recently could be given of the haemoglobin in muscle comes very near to the idea of a store. The occurrence of haemoglobin is characteristic of those muscles which undergo rhythmic contractions over long periods of time—such are the heart muscle of the larger mammals and the wing muscles of birds. Now it is of the nature of a muscle that when it contracts it should require more oxygen; on the other hand its supply is temporarily cut off because the pressure of the contracting fibres pinches the blood vessels. This has been beautifully shown by Anrep and his co-workers (1927) for both the heart and skeletal muscle. The actual internal medium of the contracting muscle would suffer great oscillations in the content of dissolved oxygen. But by enriching that medium with a copious supply of haemoglobin the contracting elements can be supplied with a continuous supply of oxygen, even though this comes from a discontinuous source.

More recently another rôle has been assigned to muscle haemoglobin by Brinkman and Margaria (1931), namely that of a catalyst which facilitates the dissociation of CO_2 from, and its association with, the bicarbonate present.

Yet when we have given what credit we can to the properties of haemoglobin there remains the fact that the "flat" part of the curve is only a third of the whole. If the ordinary utilisation of oxygen reduces the saturation of the haemoglobin from 97 to 65 per cent., that utilisation could be doubled, but not more than doubled, without greatly reducing the oxygen potential of this plasma.

In point of fact the oxygen requirements of the muscle during exercise are much more than double that during rest, and therefore additional machinery is necessary if the level of oxygen in the plasma of the venous blood is to remain in the region of 34 ± 7 mm. The method on the larger scale of preserving the constancy of the oxygen content of the venous blood is the supply to the tissue of a more copious flow of blood, involving as it does the alteration in calibre of the vessels which irrigate the tissue: involving also consequential alteration in the rate of the heart, the quantity of blood in remote organs, and many other things. Into the detail of these alterations it is not my purpose here to enter. I wish to point out that we have taken the same jump as in the consideration of the higher regulation of respiration and of temperature, the jump from a purely chemical and palliative process to one of elaborate nervous complexity, which primarily involves the sympathetic system.

V. BLOOD SUGAR.

The blood sugar in the lower vertebrates is much more variable than in man. In other words the internal "milieu," so far as glucose is concerned, exhibits much less "fixité."

Observations in fishes have been made by Hall and Gray (1929), who have kindly furnished me with the following data:

Goose fish	0 –10	mg. sugar per 100 c.c. blood
Scup	35·3–116·2	„ „

So much for cold-blooded animals; in ducks Seitz (1929), working in the North-Western University, Chicago, finds the normal range of blood sugar to be 100–160 mg. per 100 c.c. of blood. Chickens appear to be even more variable. Koppanyi, Ivy, Tatum and Jung (1926) give the normal maximum as 200–350 mg. glucose per 100 c.c. of blood. Holmes and Holmes (1927) give a few figures for normal mammals:

Cats (3)	123, 110 and 118 mg. per 100 c.c. blood
Rabbits (2)	160 and 150 „ „

The percentage of sugar in human blood is kept at a remarkably constant level. As in the normal man no appreciable quantity of sugar is secreted in the urine, it follows that approximately all the carbohydrate eaten is sooner or later oxidised. In the meantime it is stored in the liver and in the tissues. The constancy in the blood is then assured by:

(*A*) The abstraction of sugar from the blood if an excessive amount is thrust into it as after a carbohydrate meal. This excess is taken up by (1) the liver, and (2) the muscles.

(*B*) The contribution of sugar to the blood from (1) the liver, and (2) the muscles, where this or that tissue oxidises the store and depletes the blood to make good that store. Working backwards through the statement which has just been made, we are faced with the following situation.

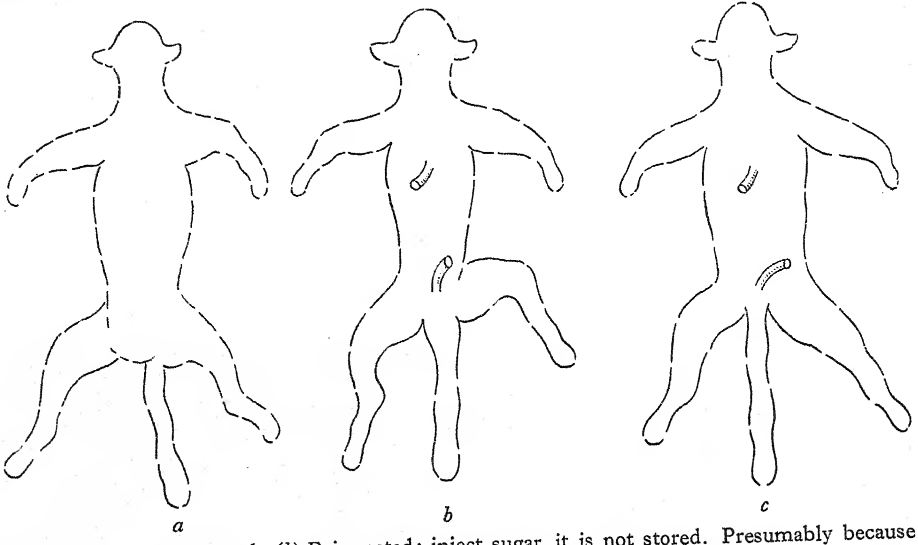
(*a*) *The replacement of the store of glycogen in muscle requires the immediate presence of insulin.* In an eviscerated animal if sugar be injected into the blood in the absence of insulin it is not taken up by the tissues, but if sugar plus insulin be injected 90 per cent. of the sugar can be accounted for either as glycogen stored or sugar oxidised. See Fig. 52.

(*b*) *The store of glycogen in muscle is unreplenished unless the vagi are intact.* If the sciatic nerve on each side be cut in the cat, and if the distal end of the nerve be stimulated the muscles supplied by the nerve lose most of their glycogen in a matter of minutes consequent on their contraction. In the spinal animal, or the animal with cut vagi, the loss is not made good, but in the intact animal or even the decerebrate animal the glycogen store in the recently stimulated muscle is replenished more or less completely in about an hour.

(*c*) *The acquisition of glycogen by the tissues is controlled by the immediate vagal secretion of insulin* from the pancreas into the blood is the conclusion drawn by

Hoet and his colleagues (1930) from the above observations ((*a*) and (*b*)), and hence that the storage of sugar is as directly, as say, respiration, the manifestation of the activity of the central nervous system.

(*d*) This would appear to be true not only of the storage of carbohydrate in muscle following the depletion of its glycogen store, but of the storage of glycogen in the liver as the result of hyperglycaemia, for if in the decerebrate cat sugar be injected into the blood it is absorbed from the circulation only if the vagi be intact.



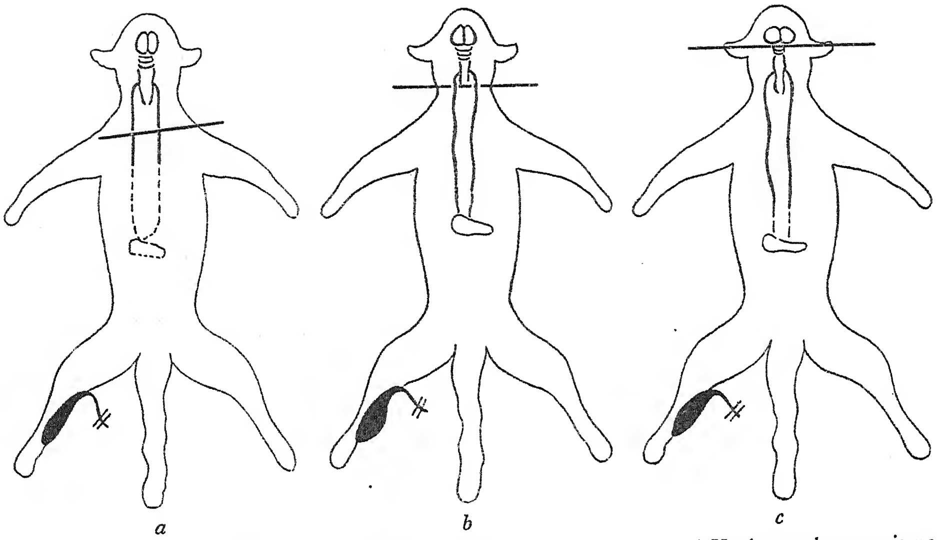
(*a*) Inject sugar, it is stored. (*b*) Eviscerated: inject sugar, it is not stored. Presumably because insulin lacking. (*c*) Eviscerated: inject sugar + insulin, sugar stored.

Fig. 52. Conditions governing the picking out of sugar from the blood.

VI. GENERAL DISCUSSION AND CONCLUSIONS.

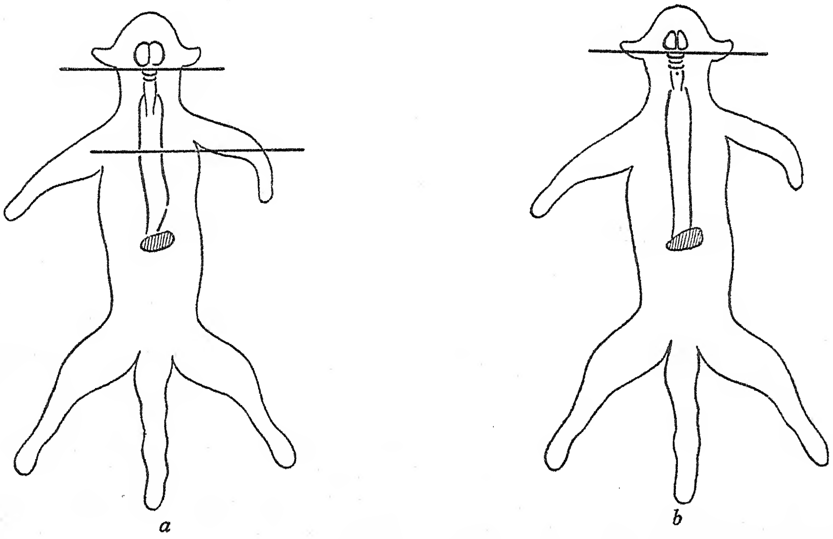
Now I must turn for a moment to the consideration of what to me is the most interesting aspect of Claude Bernard's famous statement: "The fixity of internal environment is the condition of a free life." Authors of great distinction have emphasised the importance of the statement, they have stressed the fixity of the environment, but there is a curious silence about the freedom of the life. What has the organism gained by constancy of temperature, constancy of hydrogen-ion concentration, constancy of water, constancy of sugar, constancy of oxygen, constancy of calcium and the rest? Is not the poikilothermic animal a very good animal? The bass or the perch with a blood on the acid side of normal, what fault is to be found with its muscular contraction or its heart beat? You may take other organs of the body and subject them to a concentration of oxygen far below that necessary for man as a whole and they will function excellently. What then is this free life of which the fixity of internal environment is the condition?

One approach to the investigation might be to consider what happens to man if the higher or lower limits of the fixity are passed over.



Stimulate sciatic nerve till glycogen in gastrocnemius has disappeared. (*a*) Vagi cut, glycogen is not restored. (*b*) Spinal animal, glycogen is not restored. (*c*) Decerebrate animal, glycogen is restored.

Fig. 53. Factors necessary for storage of carbohydrate in muscle.



(*a*) Decerebrate: vagi *cut*, sugar injected *not* taken up by liver. (*b*) Decerebrate: vagi *intact*, sugar injected taken up by liver.

Fig. 54. Conditions necessary for storage of carbohydrate in liver.

A brief statement of the results will be found in Cannon's (1929) article on homeostasis. This I have taken as the basis of the following table, in which the data are either taken directly from Cannon or from the references which he gives, except in a few cases when the information was deficient.

Glance your eye down this list and tell me what these symptoms have in common. Possibly my mind reacts to them rather more rapidly than that of some because it has been my fate to have experienced a number of these deficiencies and excesses in my own person. The recollections which such experiences recall are quite clear cut.

Environment	Deficient	Excessive
Temperature	Inertia	Delirium
Oxygen	Unconsciousness	—
*c*H	Headache	Coma
Glucose	Nervousness Feeling of "goneness" Hunger	—
Water	(Weakness, Asher)	Headache Nausea Dizziness Asthenia Inco-ordination
Sodium	Fever	Reflex irritability Weakness Paresis
Calcium	Nervous twitchings Convulsions	Apathy Drowsiness verging on coma General atonia

What comes back when I recall the attempt to reduce my body temperature? About the effects on the heart I have told you; they were interesting but in no way arresting, but what comes back is the effect on my mind. In each of the two experiments which I performed there was a moment when my whole mental outlook altered. As I lay naked in the cold room at Woods Hole I had been shivering and my limbs had been flexed in a sort of effort to huddle up, and I had been very conscious of the cold. Then a moment came when I stretched out my legs; the sense of coldness passed away, it was succeeded by a beautiful feeling of warmth: the word "bask" most fitly described my condition; I was basking in the cold. What had taken place, I suppose, was that my central nervous system, or at least the subthalamic portion of it, had given up the fight, that the vaso-constriction had passed from my skin, and that the blood returning thither gave that sensation of warmth which one experiences when one goes out of a cold-storage room into the ordinary air. I suppose too, that had the experiment not ended at that point my temperature would have fallen rapidly and that I was on the verge of the condition of travellers when they go to sleep in extreme cold never again to awake. And I was conscious of other reversions of mental state; not only was there a physical extension of the limbs, but with it came a change in the general mental attitude. The natural apprehension lest some person alien to the experiment should enter the room and find me quite unclad disappeared—just as flexion was changed to extension, so the natural modesty was changed to—well I don't know what. Clearly one should be very cautious about taking these liberties with one's mind—and that is the point, the higher parts of the central nervous system were the first things to suffer.

So much for cold and now for heat. What happens if the body temperature rises? I suppose there are few of my readers who have not at one time or other experienced this variation in environment—the result is delirium, before the heart has lost its efficiency or the respiration is more than quantitatively affected the coherence of the mind has gone. In the case of many fevers the effect of toxins must be eliminated before any conclusions can be drawn, but in heat stroke no such considerations come into play.

Heat stroke. Little systematic work appears to have been done with regard to the relative effect of heat in the mental processes of man and of animals. Rice and Steinhaus (1931) caused dogs to swim in water ranging from 15° C. to 40° C., and so produced a variation in the body temperature between the limits of 35° C. (95° F.) and 43° C. (109° F.) respectively. As regards the mental condition of these animals no explicit statement is given but the following sentence perhaps throws a sidelight: "Complete fatigue was considered achieved . . . when his movements were insufficient to keep his head above water." Clearly, although the mentality may have deteriorated the dogs were conscious and sane (if I may use the term). The same would not have held of man, even had he been alive.

I am indebted to Dr Frank Marsh, author of "Etiology of Heat Stroke and Sun Traumatism" (1930) for some information about the effects of high temperatures on rabbits. He writes: "The rectal temperature rose to between 109·5 and 112° F. in the course of three-quarters of an hour. During the period when the temperature was rising there were few symptoms. . . . Over 107° F. the rabbit's face assumed a peculiar anxious expression, it was not unconscious but supremely indifferent, it took no notice of water when offered to it although it was obviously thirsty. . . . Somewhere between 108° F. and 110° F. most of the rabbits lost consciousness."

From the paper named above (Marsh (1930)) I quote: "The experimental heat hyperpyrexia observed in rabbits very closely simulated the 'heat stroke' observed in man in this country (Iraq) and elsewhere.

"The nervous system of the rabbit resists heating to 109° F. for 1¼ hours. The temperature of collapse varied slightly in different rabbits, in eleven experiments it equalled or exceeded 110° F. Man usually collapses at 104° F. or 105° F."

Probably the literature would provide other and abundant information calculated to show within how narrow a range of temperature the mental processes of man are restricted, as compared with the more simple mental processes of the lower animals (but not much lower).

So much for temperature.

Hydrogen-ion concentration is a quality of the blood which may be reduced with little difficulty; it is only necessary to breathe with sufficient violence for a sufficient length of time. Try to pant as violently as you can for three minutes and if you are not "fuzzy in the head" at the end of that time I shall be greatly surprised.

This question arises, however: Is the fuzziness the direct result on the cerebrum of the washing out of CO_2, or is it due to cerebral anaemia secondary to a general fall of blood pressure?

Increase of hydrogen-ion concentration. The simplest way of increasing the hydrogen-ion concentration of the blood is the inhalation of an atmosphere which contains an abnormally high percentage of carbonic acid. The highest percentage which I know to have been breathed experimentally was eleven, and that for a short time by Mr J. B. S. Haldane. Dr Margaria and I have been in an atmosphere of 10 per cent. CO_2 in air for perhaps 5 min., but I think that somewhere around 7 or 8 per cent. is as much as can be withstood for any extended period—and with that I think Mr J. B. S. Haldane would agree. Margaria and I spent about 20 min. in 7·2 per cent. CO_2, and were quite ready to come out at the end. Our symptoms were rather different, but in both cases they were connected with the highest parts of the central nervous system. Margaria suffered from headache for the rest of the day, and Haldane was affected in the same way: my own symptoms were no less definite, but were those of mental fatigue—inability to concentrate on or even listen to conversation without an effort, the tendency to take up a newspaper, read a few lines of one paragraph, preferably something quite unimportant, then a few lines of another without finishing anything, and so forth. This was associated for a few hours with a feeling which was not exactly hunger and not exactly nausea, but a sort of mixture of the two: the mental symptoms lasted about 2 days. I took some samples of my own alveolar air in the chamber at intervals. Analysis proved that there had been errors of manipulation in the two last samples. Now the interesting point is not that these errors occurred, though that is quite significant, but that I could have gone into a court of law and sworn that one at least of the two was correctly taken. On the occasion on which we were in 10 per cent. CO_2 I was, when I came out, retaining my grip of things only with an effort. Margaria and I agreed on two things, firstly that we did not want to repeat this experiment unless there was some good reason for doing so, and secondly that our reluctance was due to our unwillingness to expose the higher parts of our brain to the influence of so much carbonic acid.

Oxygen want. The symptoms of oxygen want are too well known to need much recital. Such as they are I have experienced most of them.

(1) *Acute.* In its most acute form want of oxygen first affects the reasoning portions of the brain; if persisted in unconsciousness follows. My mind in this connection reverts to an experiment which I was doing in company with Alfred Redfield. I was on a bicycle ergometer, and certain manipulations of taps were required of me. I was breathing nitrogen, or at least some mixture poor in oxygen. When the time came to turn the taps I made no movement. Redfield at once realised that my mind had become too confused to know what to do, and directed me to carry out each process. Under his direction there was no difficulty in observing the ritual.

Many other instances might be given of similar lapses. One of the most striking is that in which the late Sir Clement le Neve Forster, partially overcome with carbon monoxide in a mine, sat committing to paper lengthy farewells to his family when he knew that he had only to get up and walk about 20 yards to be in a place of safety.

(2) *Chronic.* The more chronic forms of oxygen want have been the object of abundant study. It is only necessary to say that those which beset the dweller at high altitudes are symptoms of the brain. They may be referred to other parts of the body, palpitation of the heart, breathlessness, vomiting, etc. A moment's consideration shows that breathlessness is not an affection of the chest, but of the nervous mechanism which operates the diaphragm and the intercostal muscles.

Oxygen want, as has been shown by Greene and Gilbert (1921), never accelerates the perfused heart. Its action is in the opposite direction, and in order to affect the perfused heart a degree of anoxaemia is required which the brain would never tolerate. The palpitation induced by exercise at high altitudes is nervous.

Apart, however, from these features there are others affecting higher parts of the brain. I remember one of our party saying after we had left Orotava: "Most of us suffered from mountain sickness, we were all interested in its relation to oxygen want, we all knew that there was an abundance of oxygen cylinders at hand, yet none of us thought of trying to see whether oxygen inhalation would rid us of our symptoms—that was characteristic."

Oxygen excess. It seems doubtful whether the effects of exposure to excessive concentration of oxygen can rightly be discussed in an article on the evolution of the constancy of the internal environment. For my reticence there are two reasons. The first is that as the organism has never in its evolution had to meet an excessive pressure of oxygen such a contingency can have no place in evolution. The second reason is that oxygen being something which is inhaled is, in its relation to the lung, part of the external environment, and the fact that it produces pneumonia in rats and mice might be deemed irrelevant.

Yet with these reservations it may not be amiss to record the little that is known concerning the effects on the organism of a too high concentration of oxygen in the blood. They were first discovered by Paul Bert (1878), and have been confirmed so far as some of the meaner creatures are concerned by Lorrain Smith (1899). With regard to man there is no information on the subject.

Two larks were placed in a chamber, and the oxygen raised to a tension of 301·4 per cent. of an atmosphere. They at once became excited, and moved rapidly about the chamber. After 13 min. exposure to this tension, they were simultaneously thrown into violent convulsions. These recurred at short intervals. They began to subside in about an hour. After 2 hr. 7 min. the chamber was opened. One of the birds remained in an unconscious condition, with occasional epileptiform convulsions, for about 1 hour after, when it died. The other survived, and was very active and restless for a while, but became very sluggish later. When it was fed by the hand, however, it shook off its drowsiness for a short time, and again assumed its normal activity. It survived in this condition for several days.

Normality then takes place between limiting concentrations of a number of materials or between limiting physical conditions. If the limits are transgressed something happens to impair the efficiency of the organism. Look down the list of disabilities produced by alterations in the internal milieu (p. 82) and you will see almost no reference to the grosser bodily functions, nothing about muscular contraction as such, nothing about the heart as such, nothing about the kidney, the liver or the pancreas. In almost every case the blow is to the nervous system; we can go further, in almost every case it is to the central nervous system; in almost every case it is to the higher parts of the central nervous system. And so far as our investigation has shown us anything it has shown that the fixity of internal environment is controlled by the upper part of the central nervous system, and it is as a general rule the upper part of the central nervous system which suffers if the environment alters beyond physiological limits. The fixity of the internal environment is the condition of mental activity.

Each century, and now each decade, add emphasis to the antithesis between the complete insignificance of man when considered as part of the material universe and the astounding ascendency to which his intellect has attained in comprehending the universe in which he is placed.

Of that intellectual ascendency, "la fixité du milieu intérieur" appears to be the, or at least a, condition; of that intellectual ascendency—"la vie libre" is no inapt description.

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ROCK-BURROWING ECHINOIDS

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I. INTRODUCTION.

IN the echinoids, the habit of burrowing into rocks occurs entirely as a protection against the environmental factors wave and tidal action, and in consequence is only found in localities where these influences are at times excessive, and then only if natural cavities in the rocks, giving the necessary protection, are absent. No species of echinoid has yet been discovered which is universally dependent on this habit for its existence, and in this the echinoids differ from the more specialised rock-burrowing molluscs. Among the less specialised forms, however, analogies with the echinoderms occur, an example being *Petricola ochroleuca*, whose habits were noticed as early as 1818 by Lamarck (*Histoire naturelle des animaux sans vertèbres*, **5**, 443), and which is quoted as an example in this respect by Marcel-de-Serres (1856). In this species, as in the echinoids, rock-burrowing is dependent on certain environmental influences, for although, in common with other members of the genus, it normally bores into rocks, in certain parts of the Mediterranean, however, it burrows into the sand.

Strongylocentrotus (*Echinus*) *lividus* has received by far the largest share of the work that has been done on this subject. As this species is commonly found burrowing into rocks along the west coast of France, the problem has up to the present mostly been undertaken by French workers. The bulk of the work was done between the years 1854 and 1890, during which period the subject attracted great attention and eventually became the cause of almost as many controversies as in the early work on *Pholas*. The method of attacking a problem such as this and making deductions on comparatively slender evidence, which in the majority of cases often amounted to nothing more than amplified field notes, led to the formation of many theories based on pure speculation.

There are many records, other than those stated here, of echinoderms which have been found in rock cavities, but there is often so little data attached to these that it is unwise to assign to many of them the power to bore. For there is no evidence to suggest that many of these species may have ever been submitted to those environmental conditions to such an excess as to promote boring. Many may have just temporarily entered a natural crevice in the rock or a burrow which had been evacuated by its true inhabitant. John (1889) gives a list of boring species of echinoderms together with the locality and kind of rock inhabited, but this list of course needs many additions to-day.

II. REVIEW OF THE LITERATURE.

In 1827 Bennett described the rock-boring habit of an echinoid, from a species found on the coast of Co. Clare (Ireland) by J. D. Humphreys. Humphreys found these echinoids in ledges of rock along this coast in places that are never entirely exposed at low tide. The rock that is bored is presumably the millstone grit (carboniferous) of which this coast is composed. The burrows are circular and of the same shape as the echinoid, their depth being approximately two-thirds of that of the animal which they contain. Bennett states that the burrows "are large enough to admit of the animal rising in them a little, but not of its coming out easily: and their depth is in several considerably increased by the deposition, around their upper circumference, of a species of coralline (alga) several lines in thickness, and by a thin layer of which they are frequently lined throughout." The mouth of the echinoid is always downwards when in the burrow and "they adhere by their numerous suckers so firmly to the lodgments they have formed, as to be forced with extreme difficulty from them when alive." The largest individual was three inches in diameter. No theories as to how boring takes place are given. Bennett identifies this echinoid with the *Echinus saxatilis* of Linnaeus, the *Cidaris rupestris* of Lesk and the *Echinus lividus* of Lamarck (to-day known as *Strongylocentrotus lividus*). Although the specific names of Linnaeus and Lesk suggest the rock-boring habit, neither of them mention it as occurring.

Trevelyan writing in 1849 on the habits of *Echinus lividus* from Kilkee, Co. Clare (Ireland), came to the conclusion "that the animal does not possess any power, chemical or mechanical, of boring into rocks." He considered that *Echinus lividus* merely seeks out natural crevices in the rock, and states that "the bottom and sides of the cavities occupied by the *Echinus* become in time smooth and deepened, more particularly in limestone, but this I am convinced is not the effect of instinctive action, chemical or mechanical, nor of the locomotion of any one individual, but of that of countless generations which have successively inhabited the spots during the lapse of ages and have thus gradually worn the stone away and produced the remarkable appearances as of regularly bored holes, the depth of which is in many cases increased by the growth of the common Millepora (Nullipore) around them." He found young specimens of *Echinus lividus* in deserted limpet shells.

In 1854 Cailliaud in his paper on boring Mollusca devotes a short paragraph to the echinoderms, stating: "des *Echinus* rongent encore le calcaire par la voie chimique: nous avons trouvé les espèces *lividus* et *miliaris* se creusant des trous profonds dans la roche," an opinion he was to revoke the next year.

In October, 1854, Robert read a paper before the Academy of Sciences of Paris, on the rock-boring habits of *Echinus lividus* from the Bay of Douarnenez in Finistère (Brittany). The rock along this coast in which boring occurs is a hard ferruginous grit (Silurian). He considered that the echinoid definitely bores its holes, and that having once bored a burrow for itself it is unable to come out of it for the rest of its life. The bottoms of the burrows are clean and bare, and as the rock is unattacked by acids he concludes that a mechanical method of boring must be employed, and that the spines, which are turned against the bottom and sides of the burrow, are used for this purpose. Robert considered that the calcareous alga, which frequently covers the rock surface around the upper edges and the partitions between the burrows, could not possibly exist there if the animal secreted an acid for boring purposes.

Replying to Robert at the same meeting of the Academy of Sciences, Valenciennes pointed out that *Echinus lividus* is not the sole rock-boring representative of the echinoderms, mentioning as an example *Cidaris savignyi*, which bores into a coral (*Goniastrea solida*) in the Red Sea, and stressing the importance of its different systematic position from that of *Echinus lividus*. He states: "Les quelques exemples qui me viennent à la mémoire montrent qu'on trouve des espèces perforantes dans la série tout entière des espèces animales, et que plusieurs d'entre elles parviennent à faire ces érosions avec les téguments les plus mous, et par conséquent les moins résistants en apparence. C'est que ces animaux (boring animals in general) usent la roche mécaniquement, par l'action de l'eau de la mer qui les baigne de toutes parts, unie incessamment au frottement de leur pied charnu (in the case of Mollusca), ou de leurs tentacules filiformes (tube-feet of echinoderms), et plus mous encore que la masse charnue des Mollusques." From the above statements it is clear that he considers that the only organs necessary to enable echinoderms to bore are the tube-feet, and that these, with the help of the sea water, gradually wear the rock away.

At a meeting of the Geological Society of France in November, 1855, a letter was read from Cailliaud to M. de Beaumont in which he mentions having found *Echinus miliaris* boring into a compact limestone in the Island of Le Four (Bay of Croisic, Brittany), also *Echinus lividus* into a hard grit from the coast of the province of Finistère (Brittany). He considers that both these echinoids bore by mechanical means, thus revoking his statements of the previous year (Cailliaud, 1854) that they bored by chemical action.

At the same meeting of this society (November, 1855) Lory read a paper on the rock-boring habits of *Echinus lividus* on the coast of the Bay of Croisic (Brittany). The rock along this coast is a coarse granite, showing great tendency to disintegrate into a sand. The echinoids only burrow when within natural pools in the rock, which contain water at low tide, and never on the outer rock surfaces. No specimens were observed that were not inhabiting burrows. Most of these natural pools are exposed to the sun, and consequently support quite a rich fauna and flora. The burrows are found closely packed together about their bottoms, a few occurring on their side walls, but always below the water level. It was found that the amount of water in some of these pools at low tide, subsequent to the occupation of the pools by echinoids in their burrows, had been permanently lowered, with the result that a circular region of old uninhabited burrows existed on the walls of the pools above the new water level. Isolated burrows were only found rarely. The individual burrows are thimble-shaped, of the same diameter as the echinoid which they contain, and average from 6 to 7 cm. in depth, the depth of any one burrow being always greater than the height of its inmate. The bottoms of the burrows are smooth, but their walls are rough with projecting crystals of quartz and felspar. Lory finds that it is difficult to remove these echinoids from their burrows without breaking some of their spines, as these are securely wedged between the projecting crystals, and considers that they are probably not able to come out of their burrows. He thinks that these echinoids do actually make the cavities in which they are found and that mechanical means must be employed, as both this granite and the grit from Finistère are impervious to chemical action. No details are given as to how boring takes place. He did not observe any boring by *Echinus lividus* in the Mediterranean in a very similar granite in the Gulf of Ajaccio (Corsica), and states: "Si réellement il s'agit de la même espèce, elle offrirait au moins une différence bien remarquable d'habitudes dans les deux mers."

At the discussion after this meeting M. Boubée remarked that this particular granite was already partially disintegrated and friable, and that the motions of the spines alone would be amply sufficient to enable the animal to bore into it. He remarked that boring had not been observed in granites that were not so altered, and considered that the absence of boring by *Echinus lividus* in the Mediterranean was probably due to differences in the hardness of the rocks on the coasts of the Atlantic and in that sea. M. Durocher also stated that not very vigorous methods need be employed by *Echinus lividus* when boring into this granite, as the direct chemical effect of the sea water on the rock would soften it to a sufficient extent, in fact the burrows may have been started by direct chemical action and only finished by the echinoid.

At the same time (1855) Deshayes severely criticised Lory's (1855) statements. The fact that the burrows exactly fit the individuals which they contain he considers is not sufficient evidence that *Echinus lividus* actually bores. He found no traces of boring on the coast of Algeria, or on many places around the coast of France, among rocks of varying hardness, composition and texture, and concludes that only natural crevices in the rock are inhabited by this echinoid. His chief reasons for considering that *Echinus lividus* does not bore are that its food consists mainly of algae incrusting the rocks and that it could not live for any length of time if imprisoned within a burrow, or on the other hand if it depended on rock boring for its existence it would necessarily have to make burrows for itself in every locality where it occurred. Deshayes considers that the buccal armature is the only organ possessed by this echinoid that could possibly make impressions on rocks, but that this has an insufficient power of movement for actual boring. Finally, he deduces, from the shape of the cavities and the presence of calcareous algae within some of them, that the animal does not bore.

In the latter part of 1855 Valenciennes proposed that the Atlantic race of *Echinus lividus*, which bores, be considered a separate species from the Mediterranean race, which does not bore. He suggests that the Atlantic form should be named *Echinus terebrans*, leaving, of course, the original name of Lamarck to the Mediterranean race. He states: "Il pourrait bien se faire qu'un examen attentif, fait sur des exemplaires vivants ou très-frais des Oursins perforants de la côte de Bretagne, démontrât que ceux-ci sont d'une espèce distincte malgré leur identité apparente avec l'Oursin de la Méditerranée." Valenciennes considers that *Echinus lividus* bores mechanically, presumably by means of the tube-feet as he stated in 1854, and agrees with Boubée and Durocher (Lory, 1855) that "le granite altéré par l'eau de mer devient plus facile à attaquer, je dirais presque à égrener."

In 1856 Marcel-de-Serres published the first of two papers also on the specific identity of the Atlantic and Mediterranean races of *Echinus lividus*, and the properties of the former race as a rock borer. Having confirmed Lory's (1855) observations that the Mediterranean race does not bore, he considers that if they are the same species, a simple change in the environment produced these two races of different habits. He mentions that the Atlantic is strongly tidal and the Mediterranean almost tideless, this giving the impression that the object of boring is not solely for protection from wave action but also against desiccation at low tide. Marcel-de-Serres gives two analogous instances in the case of Molluscs (*Pholas* and *Petricola*), in which the rock-boring habits vary according to conditions in the environment.

In 1856 Cailliaud published the first of two extensive papers on the rock-boring habits of *Echinus lividus* and *Echinus miliaris* on the coast of Brittany. Here the two species were found to bore into a hard sandstone, and into a coarse crystalline granite besides. In the granite the burrows were found situated in small natural rock pools, which were never dry at low tide (cf. Lory, 1855). All the burrows were found to be occupied, and many individuals were also found that were not inhabiting burrows, but attached to the walls of the rock pools. These echinoids, Cailliaud considers, were waiting for a burrow to become evacuated by the death or removal of its inmate. No individual was found to have settled in a burrow which was too large for it, but in one either of its own size or modified to suit itself. He considers that a burrow too large would not provide sufficient protection from wave action, but he gives no indication of what happens to those echinoids, which he says are waiting for burrows, if wave action became excessive. The smallest individuals which were found to bore were about the size of peas and stated to be fifteen days old. In spite of lengthy observations Cailliaud found no movement of the echinoid, while in its burrow, which gave any indication as to its method of boring, and in all cases the animal was found mouth downwards. From the nature of the internal surfaces of the burrow, he considers that boring takes place mechanically by means of a number of points acting as picks and concludes that the buccal armature with its five teeth is the organ that is used for this purpose. He comes to this conclusion by attempting to bore into rocks with both the spines and the teeth of these echinoids. The teeth were able to withstand this treatment and made impressions on the rock surface, but the spines proved much too soft (cf. Deshayes, 1855). Cailliaud describes the method of boring as follows: the body of the animal is anchored in position by means of the tube-feet, the jaw is opened and the five teeth are protruded from the buccal chamber to the required length according to the hardness of the rock. These five teeth act together and strike the rock, dislodging fragments from it, rather than scratching it, for if scratched the sandstone would have shown the traces. If the rock is very hard the echinoid can close its jaws so as to form a bundle of the five picks. With their points thus coalesced into one, greater force is obtained. The picks being curved do not strike the rock perpendicularly but in such a way that each blow pushes aside the fragment of rock however small. The buccal armature is either moved in unison with the five picks during the striking effort, or the picks alone receive the motor impulses in their grooves and are forced against the rock and lifted again, together or separately, by their muscular system. The first method would be the more effective on account of the greater weight and power of the whole buccal armature acting together. If a particular hard object, such as an embedded quartz crystal, is encountered while boring, the picks are driven back into the muscles that work them, their elasticity thus preventing the rebound from injuring the animal. Boring into granite takes place by means of the five picks united into the form of a ram, these strike away the finer matrix around the crystals of quartz and felspar which eventually become detached whole. Cailliaud considers that *Echinus lividus* actually bores faster into granite than into limestone or sandstone, as larger fragments are detached in proportion to the time taken. On account of the flexibility of the tube-feet the whole mechanism can be orientated in all directions without their letting go of the rock, excavating thus taking place all around the burrow. As boring proceeds, the edges and points of the picks become worn down and blunted, and thus need relengthening and resharpening. Relengthening takes place by a process analogous to that of the growth of the incisor teeth of Rodents, resharpening by the echinoid rubbing the points of the worn picks against one another with powdered rock. In some specimens of *Echinus lividus* the picks were found to be no longer free but fused to their grooves and could function only as masticatory teeth. Cailliaud states that this stage is only reached in old age when boring has ceased. He found that calcareous algae, where present, only occurred on the sides of the burrow and not on the region of boring, except in burrows that had been evacuated for a long time.

In 1857 (*a*), Marcel-de-Serres, in a brief note, after examining a large number of specimens of *Echinus lividus* from both the Atlantic and the Mediterranean, disagrees with Valenciennes (1855) in considering that there is no specific distinction between any of the specimens from either sea. He states that *Echinus lividus* does actually excavate the pits in which it lives and suggests, independently of Cailliaud (1856), that the teeth and buccal armature are the organs that are used for this purpose. The Mediterranean race, he considers, does not possess the power of excavating.

In 1857 (*b*), Marcel-de-Serres amplified his statements of 1856 and 1857 (*a*) regarding the absence of specific distinction between the Atlantic and Mediterranean races of *Echinus lividus* on account of their differences in habit. Regarding the Mediterranean race he states: "Les Oursins de la Méditerranée peuvent, du reste, se

passer de creuser des cavités pour s'y loger, habitant une mer sans flux ni reflux, généralement plus calme et peu tourmentée. Ils n'éprouvent donc pas, dans de pareilles circonstances, le besoin de se mettre à l'abri de la fureur des flots. Quant à la même espèce de l'Océan, vivant dans une mer plus agitée, il est tout naturel qu'elle cherche à se défendre contre les brisants des vagues ou à ces alternatives, non moins à craindre, de se voir plongée dans l'eau de la mer, ou mis à sec sur le rivage. Il y a donc en quelque sorte nécessité pour les uns de se loger dans l'intérieur des roches, tandis que les mêmes besoins n'existent pas constamment pour les autres. On pourrait sans doute citer bien d'autres faits analogues, mais ceux-ci suffisent pour prouver que les mœurs des espèces changent, lorsque les circonstances extérieures éprouvent de notables modifications." Marcel-de-Serres mentions two instances of *Echinus lividus* boring into rocks in the Mediterranean at Bastia (Corsica) and on the Island of Planier near Marseilles, thus showing that tidal action is not the only factor governing rock-boring. Regarding the use of the buccal armature for boring he states "ces pièces (the teeth), susceptibles de mouvements variés et d'une grande extension, leur en donnent certainement les moyens," but he gives no further details of how this organ is used, and as before (1857 *a*) makes no reference to Cailliaud (1856). He states, however, that these echinoids are able to come out of their burrows at night to feed, and that the presence of encrusting calcareous algae around the burrows does not prove that they do not bore, for he finds this encrustation removed in places by the teeth of the animal.

In 1857 Cailliaud published a supplement to his paper of 1856. He states that *Echinus lividus* bores holes for itself, and is not occupying holes made by some other animal or that were due to geological agencies. After carefully studying the muscular system of Aristotle's lantern, he confirms his opinions of 1856 that the picks and buccal armature form a powerful enough weapon for boring and agrees with Marcel-de-Serres (1857 *b*) regarding calcareous algae and the reasons for the rareness of boring in the Mediterranean. On a further examination of the coast of Brittany, Cailliaud found some specimens of *Echinus lividus* inhabiting burrows 3·5 cm. in diameter at their base, but with only an external aperture of 1 cm. in diameter. He considers that these animals must have made their burrows in early youth, and have since grown. As these could never leave their burrows owing to their size, he concludes that they must rely on food that is brought into their burrows by waves and currents, and states that probably most specimens, when inhabiting burrows, may rely on these agents to bring them food, even if they are able to emerge from their holes. Having found fine rock particles between the teeth of specimens that had just been removed from their burrows, he considers that some of the material which has been bored may be passed through the alimentary canal. Cailliaud states that boring may only take place between intervals of rest during which the teeth are repaired.

In 1864 Fischer published his results of work on the rock-boring habits of *Echinus lividus* at Port Vieux, Biarritz. In this locality *Echinus lividus* is found to bore only when in large natural rock pools in a hard nummulitic limestone. Each of these rock pools was found to contain from 25 to 100 individuals, each echinoid having a burrow of its own. These basins are frequently one square metre in surface area and occur mostly between tide marks. In common with Lory (1855) he found no individuals that were not inhabiting burrows (cf. Cailliaud, 1856). The thimble-shaped burrows average from 2 to 4 cm. in depth, but cylindrical burrows up to 10 cm. deep were occasionally found. Fischer does not consider that the echinoids in these deep holes are able to come out (cf. Cailliaud, 1857). The burrows are of the same diameter as the echinoids which they contain, and from which they are very difficult to extract, they are so crowded together inside these rock basins that in places they touch each other at their peripheries. As the mouth of the echinoid is against the bottom of the burrow, Fischer finds it difficult to decide the manner in which they obtain their food. He considers that if they do not leave their burrows, their food must either be the rock which they bore away, or food material must find its way to the bottom of the burrows (cf. Cailliaud, 1857), or the echinoid must turn round in its burrow and feed at the entrance. He adds that observations on the feeding habits would be difficult as the echinoids only feed at night. Shell fragments and gravel were found at the bottom of some of the burrows, and he concludes that "ces restes donnent une idée des repas des Oursins, dont l'estomac renferme, comme on le sait, beaucoup de fragments de coquilles, de calcaires, de grains de sable et même de petits cailloux." At Port Vieux encrusting coralline algae cover all the rocks below low-tide level, and extend even on to the edges and sides of the echinoid burrows, leaving, however, a bare patch of at least one square centimetre in area at the bottom of each, corresponding to the position of the animal's mouth. The maximum thickness of this alga is only a few millimetres. Regarding the method of boring and the organs used for that purpose, Fischer finds it difficult to decide between the jaws (Cailliaud, 1856 and 1857, and Marcel-de-Serres, 1857 *a* and *b*), the pedicellariae and tube-feet (Valenciennes, 1854), and the spines (Robert, 1854). He considers that in localities where there are natural means of protection such as rock crevices, actual boring is only undertaken as a last resort, and that on sandy coasts, where there are no rocks in which to bore, *Echinus lividus* finds other means of protection such as burrowing in the sand or concealing itself under stones. He states: "La seule loi générale à laquelle ils soient soumis est la loi de conservation individuelle, et cette loi les porte à creuser leurs habitations dans des localités où tout autre genre de station compromettrait leur existence."

In 1865 Cailliaud mentions that in limestone *Echinus lividus* bores at an average rate of 1 cm. or more a year, and that it passes the material that is bored through its alimentary canal; in granite the rate of boring is quicker, but the material bored away is not ingested. As proof that boring takes place in echinoderms by the blows of the picks, Cailliaud mentions an instance of *Echinometra lacunter* which bores into basalt at St Helena. The bottoms of the burrows of this echinoid are deeply pitted, which Cailliaud considers is caused by the blows of the picks, the animal being able to orientate itself within its burrow.

In 1867 Hesse published a paper on the motives which govern the rock-boring habits of *Echinus lividus*. He states that wherever this echinoid is found to bore it is always associated with a coralline alga (*Lithothamnion polymorphum*), and that the burrows are only made within this encrustation and never in the underlying rock. He considers that the principal motive these echinoids have in boring is for feeding on this algal growth, for he does not think that they are powerful enough to penetrate the rock itself. On examining the alimentary canals of this echinoid, Hesse found calcareous material which he presumes they have bitten off, and considers this sufficient proof that they eat and digest this alga. As the alga possesses definite centres of growth from which it spreads in all directions encrusting everything in its path, he considers that the echinoid burrows owe their depth in part to the building up of the algal encrustation around the animal and not solely to its eating into it. Evacuated burrows are soon filled in by the algal growth (cf. Fischer, 1864). Hesse also found *Echinus lividus* eating into the outer layers of oyster shells, but never into the nacreous layers. He accounts for their inability to penetrate this latter region of the shells either on account of its hardness or to the absence of food material in it. He gives no indication as to the nature of the food, however, but states that the coralline alga contains more organic material (1·05 per cent.) than the outer layers of oyster shells (0·5 per cent.) and is chosen in preference to the latter. In some of the impressions formed on oyster shells, Hesse noticed grooves caused by the jaws and states, "il était facile d'apercevoir les sillons qu'avaient tracés leurs mâchoires, en se rapprochant les unes des autres dans leur mouvement concentrique, et conséquemment de constater l'action corrodante qu'elles avaient produite" (cf. Cailliaud, 1865, re *Echinometrà lacunter*). He also states that if one of these echinoids is quickly detached from its burrow the jaws are found to be arranged in the form of a cone with particles of the material that has just been bored away in between them (cf. Cailliaud, 1857). From the above statements it is clear that Hesse considers that the jaws are the chief organ used for boring, and that the method in which they are applied for this purpose is similar to their action when feeding.

Alexander Agassiz in his *Revision of the Echini*, 1872–1874, mentions four rock-boring species of echinoderms (*Strongylocentrotus* (*Echinus*) *lividus*, *Strongylocentrotus purpuratus*, *Echinometra van brunti*, *Cidaris thouarsii*). Regarding the habit he states briefly that "they chisel out with their teeth the solid rock by incessantly turning round and round, and keep their cave, where they are frequently prisoners for the rest of their existence, up to the size required by the growth of their test and spines by constant gnawing."

In 1880 Möbius found *Heterocentrotus trigonarius* and *Heterocentrotus mammillatus* boring into dead coral limestone in Mauritius in a comparatively sheltered part of the reef. The echinoids are imprisoned in burrows, which are larger in diameter below than above, and he considers that food must be carried to them by current and wave action (cf. Cailliaud, 1857). He found foraminifera in their alimentary canals. Möbius states that boring takes place by means of the two kinds of spines that are found in this genus, and that these scrape away the rock, and the deeper the burrow the more the long triangular sectioned spines around the upper part of the animal are brought into play.

In 1889 John published an extensive paper on the rock-boring habits of *Strongylocentrotus* (*Echinus*) *lividus*, *Sphaerechinus granularis* (*Echinus brevispinosus*) and

Arbacia pustulosa, which were found by Simroth in basaltic lavas between tide marks on the island of San Miguel (Azores). The rocks here are covered by a layer of coralline algae, *Lithothamnion polymorphum* and *Lithothamnion cristatum*, leaving little bare surface exposed. The burrows of these echinoids vary in size from 2 to 10 cm. in diameter, their depth being always greater than the height of their inhabitant. The burrows exactly fit the animals which they contain, and from which they are very difficult to extract, their spines being securely wedged between the irregularities of the walls of the burrows (cf. Lory, 1855). The internal surfaces of the burrows are occasionally coated by a thin layer of *Lithothamnion* and sometimes show pitting at the bottom (see Cailliaud, 1865, re *Echinometra lacunter*). The entrances to most burrows are filled up by limpet shells, held in place by the tube-feet of the echinoid: these serve as an additional protection to the animal. Some of the burrows that were found were narrower in diameter at their entrance than they were below, these being apparently begun by young echinoids and enlarged later on (cf. Cailliaud, 1857; Fischer, 1864; and Möbius, 1880). In these the echinoids are of course imprisoned. John thinks that the echinoids do not move out of the normal burrows but does not definitely state that they cannot do so. He considers that if one were removed by accident another would take its place in the burrow (cf. Cailliaud, 1856). Regarding the method of boring, he states that the spines play some part, but agrees with Cailliaud (1856) that the teeth are the principal agents. John presumes that the smooth-surfaced burrows found by Trevelyan (1849) are caused by the echinoid inserting its spines into the irregularities in the walls of the burrow and setting up a rotary movement while boring with its teeth, the rotary movement being caused by the tube-feet, the spines being pressed back against one another thus polishing the walls of the burrow with their points, which eventually become worn down. During this movement John considers that the teeth, either separated or united together into a cone (Cailliaud, 1856), are pressed firmly against the rock at the bottom of the burrow by means of the tube-feet and spines, and that on the continual rotation of the body the animal wears a hole with its teeth in the rock. Any slight orientation would bring a fresh surface of the rock under its influence, thus causing the pitting which was observed. On the removal of an echinoid from its burrow, John found that the spines around the mouth were pressed up against the underside of the animal's body, and that as the animal under normal conditions is able to bear its weight on these spines when erected, it was not the weight of the animal which was flattening them, but the pressing down of the animal by means of the leverage of the tube-feet and spines against the bottom of the burrow. John repeated Cailliaud's experiments (1857) of artificially picking at the surface of the rock with the five teeth joined together in the form of a cone, and found that it took ten minutes to form a hole 1½ mm. deep by this method, but that when using a rotary movement he was able to make a hole 3 mm. deep in a few minutes. John, like Cailliaud (1856), did not observe any rotary movement of the animal when in its burrow, and concludes that this probably takes place very slowly between long intervals of rest when the teeth are repaired (cf. Cailliaud, 1857). He considers that the coarser and more porous the texture of the rock the quicker it can be bored and that this is greatly helped between tide marks by the alternate exposure of the rock to atmospheric and sea-water action which hastens its decomposition (see Durocher; Lory, 1855). Fine limestone he suggests is only able to be bored very slowly, and he agrees with Cailliaud (1856) that large crystals can be removed from granite by the wearing away by the teeth of the fine interstitial matrix surrounding them. He states that boring ceases on old age (cf. Cailliaud, 1856). John found rock fragments and pieces of *Lithothamnion* in the alimentary canals of these echinoids, and considers that these could not possibly be stray particles which had been washed into a hole already made, for they would soon be removed by wave action if the hole was for long empty. He concludes that they consist of the material which has been bored and that their presence gives additional proof that the teeth are the chief organ used for boring. He considers that the animals may digest the organic material from the calcareous algae (cf. Hesse, 1867), but that the calcareous material is unnecessary for shell formation on account of its abundance in sea water. Their main food he concludes must be brought into their burrows by waves and currents (see Cailliaud, 1857; Fischer, 1864; Möbius, 1880). John does not agree with Hesse (1867) that boring is primarily undertaken for food, and suggests that the difference in organic content between *Lithothamnion* and the outer layers of oyster shells is too small for the animal to show any preference towards one or the other. John agrees with Marcel-de-Serres (1857 *a* and *b*) regarding the factors controlling the boring of *Echinus lividus* in the Mediterranean.

In 1890 (*a*) Fewkes published his observations on the rock-boring habits of *Strongylocentrotus drobachiensis* on the coast of New Brunswick (the continuation of a brief report published in 1889). The rock in this locality is a hard mica schist forming ledges along the coast which is at times subject to a heavy surf. The boring habit of this echinoid appears to be limited only to certain individuals in exposed localities, although in many places, seemingly identical, no boring occurs. Fewkes states that "there must be certain peculiarities of environment especially adapted to these animals to present favourable conditions in individuals for this habit. There seems no satisfactory reason why, if the process of excavation is simply a habit, we should find it so rarely exhibited...We are at a loss to explain why sea urchins make excavations only when they are in certain places and under certain conditions" (cf. Marcel-de-Serres, 1856, 1857 *a* and *b* concerning the boring of *Echinus lividus* in the Mediterranean). The individual burrows are situated in small natural rock pools which often have overhanging walls (cf. Cailliaud, 1856; Fischer, 1864; Lory, 1855). The actual burrows are concave in shape, corresponding to the convexity of the animal's body, and are never deeper than the smaller diameter of the animal which they contain and are usually bare of growths internally. The diameter is always larger than that of the contained animal, thus allowing it room to move when within the burrow (Bennett, 1827). Calcareous algae (*Lithothamnion polymorphum* and *Melobesia lenormandi*) occasionally grow within the cavities and sometimes cover the rims between the burrowed pits and their outer edges, but the thickness of this encrustation does not appreciably add to the depth of the burrows affected. Fewkes finds that the bottoms of the burrows are always free from algae, but states that they might cover the whole internal surface of a burrow if it was evacuated by its inhabiting echinoid, and that a fresh inhabitant would remove this encrustation on reinhabiting it (see Cailliaud, 1856, and John, 1889). Fewkes does not know if the echinoids leave their burrows to feed and gives no indication of how food is obtained by the animal when in its burrow. He disagrees with Hesse (1867) that *Echinus lividus* is able to obtain nourishment from oyster shells, and considers that the rock fragments which have been found in the alimentary canals of rock-burrowing echinoids need not necessarily be material that has been eaten off but may be particles which have been washed into the burrows. At Grand Manan Fewkes found *Strongylocentrotus drobachiensis* boring into birch-tree posts and considers that they may obtain nourishment from these. Regarding the method of boring, Fewkes agrees with Cailliaud (1856) that the teeth are the principal organs used for this purpose, but also with John (1889) that the spines play some part. He states that "the teeth are probably chisels, which pry into the rock, or gouge out fragments, and in that way eventually remove considerable quantities of rock from its bed." Fewkes frequently found the spines around the mouth, and sometimes around the equatorial circumference of the animal, considerably worn down, and concludes that the smoothness of the burrows may be due in part to the spines and not wholly to the teeth (John, 1889). He observed no pitting on the surfaces of the burrows and states that "the surface of the depression is smooth in such a symmetrical manner that it seems necessary to suppose a rotary motion of the sea urchin to effect it" (cf. Agassiz, 1872–74; John, 1889). In common with John (1889) he did not, however, observe any rotary motion on the part of the animal when in its burrow, and concludes that if it occurs at all it is probably extremely slow. Fewkes also considers that boring may be in part involuntary, due to wave action, and that the animals, and particularly their dead shells, may play the part of stones in "pot-hole" formation where conditions are suitable. He considers that the purpose of boring is for protection against wave action and to keep water around the animal at low tide.

In 1890 (*a*) Fewkes, in connection with his theory of "pot-hole" formation, quotes some unpublished observations by Marcou, who found *Echinus lividus* boring into a nummulitic limestone at Biarritz in 1877 (cf. Fischer, 1864). The echinoids were only found to bore when within large natural rock pools, which Marcou considers are genuine "pot-holes" or "cauldrons." These "pot-holes" are from 1 to 2 ft. deep and 1½ ft. in diameter, and are of three types. Some are plain cauldron-shaped, others have a central columella of solid limestone in the middle, while in others this columella is worn away at its base and lies free in the "pot-hole." At the bottom of every "pot-hole" Marcou found small rounded stones, which he considers are for the most part the cause of their formation. A "pot-hole" contains from 40 to 60 echinoids. Each animal forms a burrow from 2 to 4 cm. deep "qu'il a creusé lui-même avec ses piquants, dont il se sert comme d'une lime." The burrows occur in definite rows one above another (cf. Fischer, 1864) on the walls of the "pot-holes" and on the columella when this is present. Marcou found no dead echinoids in any of the burrows, and the animals inhabiting burrows in those columellae, which were lying free in the "pot-holes," did not appear inconvenienced by its presumed rotation by currents. Marcou states that these "pot-holes" were formed as follows: "Des oursins s'étaient placés sur une espèce de cercle et y avaient creusé leurs niches; quelques petits cailloux detachés commençaient leur office de creusement du calcaire pour arriver à créer une station-marmite pour les échinides." The columella being untouched by the erosion of the stones and echinoid tests is thus left standing in the middle of the "pot-hole." Fewkes considers that the three types of "pot-holes" probably follow a definite sequence, beginning with those with a central columella, and ending with those in which it has been completely eroded away, but he sees no reason why these echinoids should primarily arrange themselves in a circle on the rock surface. The small stones, he considers, may have been collected by the echinoid for protection or may have been washed into their original burrows, but those found at the bottom of the "pot-holes" he concludes are probably either pieces broken from the base of the columella or its remains. The burrows in the walls and columellae of the "pot-holes" are evidently of secondary formation. In conclusion Fewkes states: "We might also add that there is evidence that the time which has elapsed between the inception of the sea urchin's work in excavation and the present condition of the cavities is probably much longer than the life of the individuals which occupy the present burrows. In an interval between two occupants the 'pot-hole' may have been enlarged by effective grinding out of the cavity by the motions of the test of the former occupant, or by fragments of the spines, the teeth, or other hard parts of the dead animal, which has left this heritage as a means, when moved by the water, to grind out the excavation for its successor."

In 1890 (*b*) Fewkes in a short note quotes a letter from Prof. E. W. Cragin regarding the boring of *Echinometra vanbrunti* and *Cidaris thouarsii* into hard igneous and metamorphic rocks at Guaymas on the coast of Mexico. Cragin found difficulty in extracting these echinoids from their burrows, and on attempting to do this states that "the echinoderms took occasion to set a number of spines against the walls of their gode (burrow) and, thus braced, could usually defy further efforts to remove them save by such harsh means as fractured the bodies of the animals." He considers that this power of holding themselves into their burrows by their spines a means of protection against wave action, but thinks that the spines would not suffer much erosion even if the echinoid was washed about freely in its burrow. Some of the spines, he states, are worn probably from this cause.

In 1911 Romanes published observations on the rock-boring habit of *Strongylocentrotus* (*Echinus*) *lividus* at Bantry Bay, Ireland. In this locality the burrows are found in a fine grained mudstone and tend to become arranged in lines parallel to the strike of the rock, which Romanes considers is due to slight differences in hardness at these places. A hard grit also occurs along this coast, but in this rock no boring occurs, the echinoids seeking out natural crevices for protection. The burrows average 3 cm. in depth and 5·5 cm. in diameter and exactly fit the individuals which they contain. Romanes is uncertain whether a burrow is formed by one echinoid or by generations inhabiting it one after another. A few deep burrows were found, in which he states the echinoids tend to regain a horizontal position after attaining a certain depth, as the sides of many of these were deeper on one side than the other. *Serpula* and polyzoan growths were found at the bottoms of some of the burrows, which he considers are evidence that boring ceases after a certain depth is obtained thus allowing these growths. Romanes found that coralline algae when present only formed encrustations of not more than 1 mm. in thickness. He considers that boring is mechanical and takes place by means of the spines, but no details are mentioned.

III. SUMMARY.

(1) *The restriction of the localities where boring occurs, and the factors governing boring.*

All echinoids which burrow into rocks do so for protection from excessive wave and current action and to some extent against desiccation at low tide. At such times the animal securely wedges itself within the confines of its burrow by inserting its spines against the walls and clinging on by its tube-feet. Burrows in rocks are only made by echinoderms in localities where suitable natural means of protection against these factors are absent (Fischer, 1864); any suitable natural crevice is made use of.

On sandy coasts, where there are no rocks in which to bore, other means of protection such as burrowing into the sand, or lying under stones, are resorted to (Fischer, 1864).

No deep-water species of echinoids have so far been found to burrow into rocks, this habit being restricted to those littoral forms whose food consists mainly of algae.

The maximum algal growth extends from high-water neap tides to a short distance below the low-water level of spring tides. Even if these echinoids inhabit the region below low-tide level it is within the range of wave action.

As all the observations so far have been made in the daytime at low tide, the echinoids have always been found stationary and securely fixed mouth downwards within their burrows. Any movement suggesting the way in which boring takes place would then not be observed (Cailliaud, 1856; John, 1889; and Fewkes, 1890 *a*). Natural pools and cavities in rocks also afford protection, especially against desiccation at low tide, and are consequently sought out by the echinoids. In certain localities powerful eddy currents may be set up in these in rough weather, with the result that the echinoids burrow into their walls for additional protection. At such times no individual that was not inhabiting a burrow could exist within these cavities.

Strongylocentrotus (*Echinus*) *lividus* is found to bore only rarely in the Mediterranean (Marcel-de-Serres, 1857 *b*). As the tide in this sea is negligible, boring occurs as a protection against wave action, and then only in localities where natural means of protection are absent. Compare the peculiar restriction of boring of *Strongylocentrotus drobachiensis* on the coast of New Brunswick (Fewkes, 1890 *a*).

The peculiar "pot-holes" found by Marcou (Fewkes, 1890 *a*) at Biarritz are abnormal structures and have not been caused by the boring of *Strongylocentrotus lividus*. There is no evidence to suggest why these echinoids should arrange themselves in a circle on rock surfaces.

(2) *The structure of the burrows.*

The rock burrows of echinoids wherever found are of the same general structure regardless of the species or the kind of rock concerned (Fischer, 1864). The burrows are thimble-shaped and circular in diameter, and differ from each other only as regards depth.

Burrows of three different relative depths occur:

(*a*) Shallow burrows, in which the depth is less than the height of the inhabiting echinoid (Bennett, 1827; Fischer, 1864; Fewkes, 1890 *a*; Marcou (Fewkes 1890 *a*); Romanes, 1911).

(*b*) Burrows of medium depth, in which the depth is the same or slightly greater than the height of the contained individual (Lory, 1855; John, 1889).

(*c*) Very deep flask-shaped burrows, in which the diameter at the entrance is less than that at the bottom or of the inhabiting echinoid (Cailliaud, 1857; Fischer, 1864; Möbius, 1880; John, 1889; Romanes, 1911).

There is no evidence to show that the echinoids are imprisoned within the shallow or medium depth burrows (Robert, 1854; Lory, 1855; Agassiz, 1872) for they emerge at night to feed at suitable states of the tide (Marcel-de-Serres, 1857 *b*), returning when necessary to the same burrow. The inhabitants of the very deep burrows are of course imprisoned within them.

At certain seasons of the year many littoral echinoids, including species that burrow into rocks, forsake their burrows between tide marks and migrate into deeper water. Thus many of the burrows of shallow and medium depth may only be occupied during certain periods. This migration is stated by Cailliaud (1857) to occur on the coast of Brittany in the case of *Strongylocentrotus lividus*.

In some cases the present inhabitant of a burrow may not have contributed anything towards its formation. Burrows once formed would naturally be utilised by successive generations of echinoids if still suitable for habitation.

One of three things might happen to an echinoid when taking up its abode in a burrow:

(*a*) It may settle within a burrow which is too large for it to wedge itself in, in which case it may be washed out and perhaps killed.

(*b*) It may find one in which it could securely fix itself and which it would enlarge as it grew during its period of occupation.

(*c*) It may have to make a new burrow.

The ultimate fate of all burrows is to be enlarged beyond the maximum size required for even the largest echinoids, and eventually, if vertical or suitably inclined, to become collectors for small stones, which will in time assist to convert some of them into "pot-holes."

Calcareous algae and other stony growths soon fill in any burrows that are evacuated for any length of time (Cailliaud, 1855; Fischer, 1864; Hesse, 1867;

Fewkes, 1890 *a*), making them smaller in diameter and shallower. This tends to keep them within the range of sizes required for the echinoderms.

The burrows lined by *Lithothamnion* found by Cailliaud (1856), Fischer (1864), Fewkes (1890 *a*), and John (1889), and the impression that Hesse (1867) obtained that *Strongylocentrotus lividus* only bored within the thickness of the encrusting calcareous alga, can be accounted for by an echinoid reinhabiting an already partially filled-in burrow.

The very deep flask-shaped burrows must have been formed by the same echinoids which they now contain, having been deepened and widened as growth of the animal took place (Cailliaud, 1857; John, 1889). These burrows must have been caused, either by a prolonged period of imprisonment of the echinoid within it, or by an excessively rapid growth of calcareous alga around the entrance of the burrow. In old individuals the motion of the animal within the burrow may not be sufficient to prevent calcareous algal growths from completely enclosing the echinoid. In certain localities where food can be easily obtained by these imprisoned individuals, the deep burrows may have been formed naturally, the echinoid having no necessity to emerge (Möbius, 1880).

(3) *The method of boring.*

Echinoderms burrow into rocks mechanically by means of their spines and teeth, or by their spines alone. Only Cailliaud (1856) and John (1889) suggest in any detail how this takes place.

By experiment John (1889) proved that his theory of boring was more successful than the method suggested by Cailliaud (1856), but in some species a combination of both methods may occur. Cases when completely fresh burrows have to be made are rare, otherwise boring enlarges a pre-existing burrow on the growth of the animal and mostly is due to the action of nestling into the burrow after emergence.

A screw-like rotary motion is almost certainly employed by an echinoid, when settling within its burrow, during which movement the spines may force the teeth against the bottom of the burrow.

In the formation of a burrow the spines accomplish the widening; the teeth, and to some extent the spines around the oral pole, the deepening. The teeth may, however, play an insignificant part in deepening a burrow that has once been modified for habitation. In these cases the slight deepening necessary on growth takes place mostly by means of the oral spines, smooth-bottomed burrows as found by Lory (1855) and Fewkes (1890 *a*) resulting; necessary widening takes place by means of the spines around the equatorial plane of the animal.

In the case of a burrow in process of formation, or on reinhabiting one that needs deepening, the echinoid can orientate itself sufficiently within it so as to bring different areas of the bottom of the burrow under its teeth, causing the pitting observed by Cailliaud (1865), Hesse (1867) and John (1899).

A fresh burrow can only be started on the site of a small natural hollow on the rock surface which affords sufficient purchase for the spines and tube-feet, and the softest areas of the rock are naturally chosen (Romanes, 1911).

During active boring, such as in the making of a new burrow, an echinoid is only able to work between intervals of rest when its teeth are regrown and sharpened (Cailliaud, 1856). If wave action suddenly becomes excessive during this period, only those echinoids which have succeeded in digging themselves in sufficiently for protection before their teeth have become so worn as to be inefficient for boring survive; the others will be washed from their foothold.

Coarse-textured rocks can be burrowed into quicker than rocks of fine texture, according to Cailliaud (1856) and John (1889), but much depends on their degree of disintegration (Boubée and Durocher, see Lory, 1855; Valenciennes, 1855).

The tests and their broken fragments, the teeth and buccal armatures of dead echinoids, if not washed out of the burrows, may for a short time help in the erosion of the cavities like stones in the formation of "pot-holes" (Fewkes; and Marcou, 1890 *a*). These fragments, however, are too light and soft to make much impression on the burrow.

It is not possible for any living echinoid when within its burrow to become an involuntary agent in boring by being whirled around inside it by wave and current action as suggested by Fewkes (1890 *a*). The echinoids securely fix themselves within their burrows to withstand this very action as well as from being washed out.

There is every reason to suggest that active boring ceases with old age (Cailliaud, 1856; John, 1889). Old individuals probably remain for the most part in their burrows and do not move out of them.

(4) *Feeding in relation to rock burrowing.*

Echinoids do not burrow into rocks for the purpose of obtaining food. The older workers thought that limestone rock was an important article of food in the sense that it was essential for shell formation. Cailliaud, in 1857, suggested that all fragments of calcareous rocks are ingested, while those from non-calcareous rocks are not. Hesse (1867) was the first to point out the organic content of the substances burrowed into, and John (1889) that it was unnecessary for the animal to eat limestone.

The small amount of material that is bored away at any one time is either taken into the mouth and passed through the digestive tract (Cailliaud, 1857; Hesse, 1867; John, 1889), or the fragments may be taken up by the tube-feet around the mouth and passed from one to another and thus to the exterior of the burrow. In the case of those echinoids which can emerge from their burrows, any powdered residue left at the bottom of these could be removed by wave and current action when they are temporarily vacated.

All those echinoids which are unable to emerge from their burrows obtain their food by one or more of the following means:

(*a*) Food material may be washed directly into the bottom of the burrows (Cailliaud, 1857; Fischer, 1864).

(*b*) In some of the burrows it is possible for the echinoids to turn round within them so as to bring their mouths into more direct contact with any food material brought near (Fischer, 1864).

(*c*) Algae and other food may be carried by wave action on to the tube-feet around the anal pole of the animal and handed by them down to the mouth below.

Even in the case of the echinoids which are able to emerge from their holes to feed, a high percentage of their food is probably obtained by the above methods.

A certain amount of organic food material can be obtained by rock-burrowing echinoderms directly from certain limestones, particularly dead coral, this food being in the form of rock-boring algae. The organic contents of calcareous algae would also provide food material as stated by Hesse (1867).

(5) *The association of calcareous algal growths with rock-burrowing echinoids.*

The calcareous algae mentioned by most workers bear no relation whatever to the burrowing echinoids. In places where the thickness of this algal encrustation exceeds that required for the depth of the burrows, boring might take place within the depth of this alone, the burrows never reaching the underlying rock, thus giving the false impression that the alga grew up around the echinoid and enclosed it.

In some localities calcareous algae, as stated before, play a part in the structure and building of the burrows, their presence generally tending to counteract the destructive effects of the echinoids on rocks.

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RECENT WORK ON THE DEVELOPMENT OF THE VERTEBRAL COLUMN

BY E. W. MACBRIDE.

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(With 48 Text-figures.)

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I. INTRODUCTION.

THE excuse for writing the present article is the fact that during the last twelve years the development of the vertebral column in various groups of Vertebrata has been the subject of research in the Zoological Department of the Imperial College of Science. A brief summary of the results obtained and of the newer points of view which have been gained in consequence of these co-ordinated investigations is given below.

Ridewood (1920) examined the development of the column in a number of genera of Elasmobranchii; Ramanujam (1929) its development in the herring as a representative of the Teleostei; Mookerjee (1930) its development in *Triton* (*Molge*) as a representative of the Urodela and also in several genera of the Anura; Piiper (1928) examined it in the ostrich and the gull as representatives of the birds; and finally Dawes (1930) studied its growth in the mouse as a representative of the Mammalia.

We do not propose to give an historical account of all the previous researches which extended through at least fifty years; we shall take as our base line the two papers of Gadow and Abbott (1895, 1896), because these give a coherent and clear theory of the development of the column which has been embodied in text-books of zoology, a theory from which in many points the newer researches discussed in this article compel us to dissent.

Gadow's first paper deals with the development of the vertebral column in Elasmobranchs and Ganoids; his second paper describes its development in Amphibia and Amniota. According to Gadow and Abbott the notochord which is the foundation of the vertebral column in all Vertebrata receives during its development a covering consisting of two sheaths, an inner and an outer, which have quite different origins. The inner or *chordal* sheath is a cuticular product or basement membrane formed by the notochordal cells themselves. It varies in thickness and it must be permeable, as otherwise the notochordal cells would be cut off from nourishment, and in quite a number of Teleostei the notochord increases many times in diameter as the fish grows older. The outermost layer of this inner sheath is a thin hard membrane which is termed the *elastica externa*; the rest of the sheath is of a fibrous character and is termed the *elastica interna*. The outer sheath is termed the *perichordal sheath*; it is formed from loose connective tissue budded from the inner margins of the muscle segments or myotomes. Gadow was the first to show that this tissue is budded from each myotome in two places, forming two masses which he called *sclerotomes*. The perichordal sheath is formed from the innermost layer of these sclerotomes. One sclerotome arises from the myotome opposite the lower border of the spinal cord, the other is given off from the dorsal apex of the myotome opposite the upper border of the spinal cord. The two, however, soon fuse with each other and form one mass of loose connective tissue or *mesenchyme* in which separate dorsal and ventral portions are indistinguishable. Ridewood (1920) at my suggestion examined young embryos of the dogfish and was able to confirm Gadow and Abbott's statement. At a later period of development condensations which become converted into cartilage are formed in the continuous mass of tissue which results from the fusion of all the sclerotomes and which was termed by Gadow the *membrana reuniens*. The condensations give rise to two sets of cartilaginous arches or arcualia which abut on the chordal sheath: the upper set, termed *neural arches* or *basidorsals*, flank the spinal cord; the lower set, termed *basiventrals*, project outwards into the septa which divide the myotomes into dorsal and ventral portions. The outer parts of the basiventrals become movable on the proximal portions and form the *ribs*; but in the tail the distal portions of the basiventrals project downwards and meet in the mid-ventral line, forming what are termed *haemal arches*. The abrupt passage from ribs to haemal arches marks the spot where the trunk ends and the tail begins. Alternating with the neural arches but not extending so far dorsally are a series of smaller "arcualia" which are termed *intercalaries*. In some Ganoid fish and in some extinct Amphibia there is a series of "ventral intercalaries" alternating with the basiventrals, but these are not developed in Elasmobranchs. Finally, roofing over the spinal cord and uniting one basidorsal with its fellow on the opposite side of the animal, is a single or double series of *supradorsal* cartilages.

Gadow and Abbott explained the intercalaries as extensions of the basidorsals and basiventrals respectively round the perichordal sheath; according to them the extensions became separated from the main masses of the arcualia; thus the basidorsals gave rise to the ventral intercalaries which lay in front of the basiventrals belonging to the same segments, whilst the basiventrals budded off the dorsal intercalaries which lay behind the corresponding basidorsals. They held that the basidorsals themselves were derived from the dorsal sclerotomes and the basiventrals from the corresponding ventral sclerotomes: although as mentioned above these two sclerotomes had fused into an indistinguishable mass before the arcualia made their appearance. They did not explain the origin of the supradorsals. Elasmobranchs according to Gadow differ from all other Vertebrata except Dipnoi in that in them a perichordal sheath distinct from the rest of the membrana reuniens does not exist. The centra of the vertebrae are entirely formed from the chordal sheath—for which reason Gadow terms them *chordacentra*. This sheath becomes invaded by wandering mesenchyme cells which convert it into cartilage. The cartilage takes the form of cylinders separated by interspaces in which the sheath is transformed into fibrous tissue. In Dipnoi the sheath becomes a continuous flexible tube of fibro-cartilage and no centra are formed. In Elasmobranchs the sheath becomes partly calcified, and three different forms of calcification have been described which have been termed respectively *cyclospondylous*, *tectospondylous* and *asterospondylous*. The cyclospondylous calcification consists in a single cylinder of calcification inside each centrum. In the tectospondylous mode of calcification there is a concentric series of calcified cylinders in each centrum. Finally the asterospondylous calcification consists of a series of radiating wedges, appearing in section like the spokes of a wheel, with the notochord in the centre. Gadow admits, however, that in some Elasmobranchs the expanded ends of the dorsal and ventral arches meet each other outside the chordacentrum and thus constitute an external ring of cartilage which is not derived from the chordal sheath.

Amongst the cartilaginous Ganoids (*i.e.* the sturgeon family) there is a thick chordal sheath which does not become converted into cartilage and on the outside of which the bases of the arcualia rest. In all the bony fish, including under this head the bony Ganoids (*Amia* and *Lepidosteus*) as well as Teleostei, a new type of vertebra, the so-called *arcocentrum*, makes its appearance. According to Gadow and Abbott in these fish the chordal sheath is not invaded by mesenchyme cells and the centrum lies entirely outside it and is formed entirely from the basal ends of the arches. In *Amia* and *Lepidosteus* the basidorsal buds off cells which grow downwards round the chordal sheath and the basiventral buds off cells which grow upwards; these two masses of cells meet and form a ring which becomes ossified and forms the arcocentrum. Owing to the backward slope of the myotomes as they descend from the mid-dorsal line, the basidorsal of segment 50 lies above the basiventral of segment 49 and fuses with it. Thus the arcocentrum comes to lie across the septum between two myotomes, and these myotomes therefore become attached to the centrum before and behind. In the tail of *Amia* there are two bony centra corresponding to each myotome, and to one of them both the neural and haemal arches are attached. This circumstance created a difficulty for Gadow, because he was bound to assume that one of the bony rings had been formed by an upgrowth from the basiventrals, and to this ring the haemal arch which is an outgrowth from the basiventrals should have been attached: he assumed therefore that this arch had secondarily become shifted, an hypothesis which creates serious difficulties.

Amongst Amphibia and Amniota Gadow and Abbott found the arcocentrum to be of universal occurrence. In all of these animals the true chordal sheath remains thin and is never invaded by cells and the whole vertebra is formed outside it.

Amongst Urodela in the tail region basidorsals and basiventrals and in addition dorsal and ventral intercalaries were developed. The centrum was formed as in bony Ganoids by the union of basidorsals and basiventrals, whilst the dorsal intercalaries (or interdorsals) of segment 50 united with the ventral intercalaries of segment 51 to form an intervertebral cartilaginous disc which constricted the notochord. This disc subsequently became cut into anterior and posterior portions, with a joint between them. The anterior portion became added to the vertebra in front, whilst the posterior portion joined the vertebra behind. In the trunk region the basiventrals were absent and the centra were totally formed by the basidorsals: the intervertebral discs, however, were formed in the same manner as in the tail. Amongst Anura, according to Gadow, not only basiventrals but also ventral intercalaries were absent, and consequently the intervertebral discs were formed from the dorsal intercalaries only.

In Amniota (reptiles, birds and mammals) Gadow and Abbott found that although the vertebrae were arcocentra yet these were constructed in a totally different manner from those of Amphibia, so that the resemblance between the two kinds of vertebrae was superficial only. In Amniota dorsal intercalaries were absent, but ventral intercalaries were large and gave rise to the entire centrum, whilst the basidorsals only produced the neural arches which were united by suture to the centrum. The basiventrals to which the heads of the ribs were attached formed intervertebral cartilaginous pads, except the first pair, which united to form the ventral portion of the atlas ring. The centrum corresponding to this became attached to the second centrum as the odontoid process.

II. PISCES.

1. Elasmobranchii.

We may now discuss the modifications of Gadow's theory rendered necessary by the researches carried on in the Imperial College of Science, and we begin with Ridewood's paper (1920) on the vertebral centra of sharks and rays. Ridewood agrees with Gadow in the assertion that each myotome produces a dorsal and a ventral sclerotome, but he insists that these two sclerotomes fuse together into a common membrana reuniens long before arcualia make their appearance, so that it is impossible to say that the dorsal sclerotomes give rise to the basidorsals. Ridewood further agrees with Gadow in asserting that mesenchyme cells penetrate the elastica externa of the chordal sheath and transform the fibrous elastica interna into cartilage which gives rise to the "primitive double cone," as Ridewood terms it, of the vertebra. This "double cone" becomes largely calcified, and thus the primitive "cyclospondylous" vertebra is formed. But Ridewood shows that in the vast majority of Elasmobranchs the primitive cone forms an insignificant part of the fully developed vertebra. Between the bases of the arches are masses of connective tissue derived from the innermost portion of the membrana reuniens which become converted into cartilage. These masses are termed by Ridewood *intermedialia*, and they correspond exactly to what is called the perichordal sheath in other vertebrates.

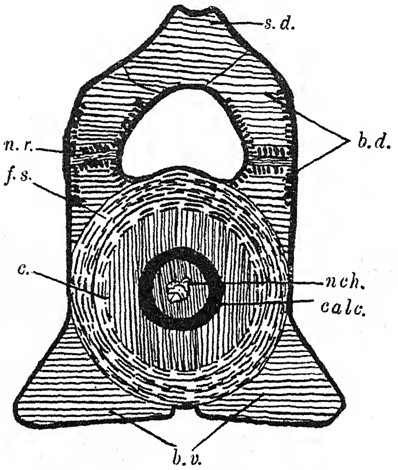

Fig. 1.

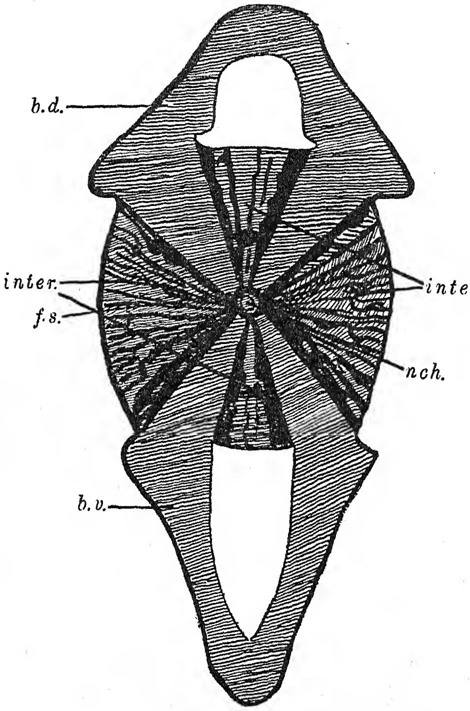

Fig. 2.

Fig. 1. Transverse section of a vertebra of *Heptanchus* from the middle of the trunk. In this shark the whole of the centrum is formed from the notochordal sheath. *b.d.* basidorsal; *b.v.* basiventral; *c.* fibro-cartilaginous centrum; *calc.* calcified ring within the centrum; *f.s.* fibrous sheath of centrum; *nch.* remains of notochord; *n.r.* aperture in the basidorsal through which the spinal nerve passes; *s.d.* supradorsal cartilage.

Fig. 2. Transverse section through a caudal vertebra of *Carcharodon*. In this shark there are enormous arcualia resting on a thin sheath of a slender notochord. In this sheath is the primary calcified cone but a huge secondary growth is added to the primary sheath in the form of four large intermedialia between the bases of the arches. In these intermedialia are "asterospondylous" calcifications indicated by radiating black lines. *b.d.* basidorsal; *b.v.* basiventral; *f.s.* primary sheath of the notochord; *inter.* intermedialia; *nch.* remains of the notochord.

Only in the most primitive sharks is the cyclospondylous condition retained. It is found for instance in *Heptanchus* and *Chlamydoselache* which belong to the family Notidanidae (Fig. 1). In *Heptanchus* the whole centrum is formed from the enlarged and thickened fibrous chordal sheath in the centre of which lies the shrunken remains of the notochord. Long cylindrical centra are only developed in the tail region. As we pass forwards into the trunk region, they become shorter and shorter in an antero-posterior direction separated by longer and longer tracts, in which the chordal sheath is transformed into fibrous tissue, till at last they become reduced to mere discs and they may disappear entirely in the branchial region. Ridewood is inclined to regard this reduction as secondary, for what reason is not clear. Van Wijhe (1922) has described the development of the vertebrae in *Acanthias vulgaris*. He asserts that the notochord becomes invested in a continuous cartilaginous tube which later becomes broken up into centra, which first appear in the tail, because the tail is functionally the most active part of the body in locomotion. If for cartilage in Van Wijhe's account we read fibro-cartilage, there will be a strong probability that his account is correct, and in this case the column of *Heptanchus* instead of representing a degenerative condition will be really primitive.

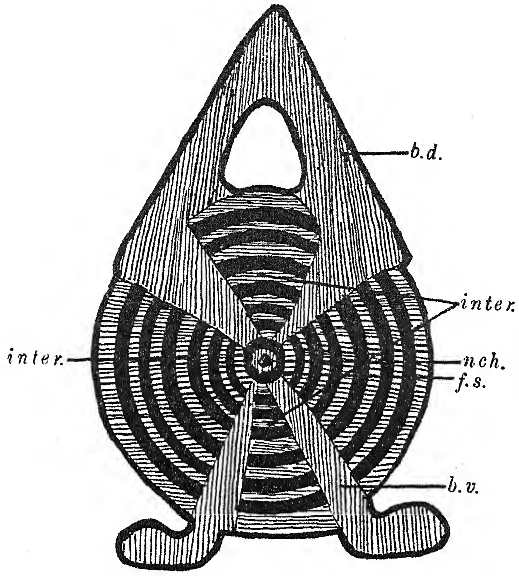

Fig. 3. Transverse section through a trunk vertebra of *Cetorhinus* in order to show one form of "tectospondyly." The remains of the notochord are surrounded by a slender sheath inside which is the primary calcified cone represented by the innermost black circle. On this sheath rest the bases of the four large arcualia. Between these bases are large intermedialia which make up the bulk of the centrum. In these intermedialia are secreted a series of concentric calcified arcs—which together make up a series of rings (coloured black) constituting tectospondyly. *b.d.* basidorsal; *b.v.* basiventral; *f.s.* primary sheath of the notochord; *inter.* intermedialia; *nch.* remains of the notochord.

When, as in most families of Elasmobranchii, intermedialia are formed, additional calcifications are formed inside them. Thus in *Carcharodon* (Fig. 2) the primitive double cone contains its "cyclospondylous" calcification. Resting on this slender cylinder, however, are four series of radiating calcified wedges developed into the intermedialia. The enlarged bases of the arches which separate the wedges are devoid of calcification. This is the typical "asterospondylous" type of calcification.

But these different forms of calcification cannot be used to define families, for the type of calcification, as Ridewood shows, may change as the vertebra grows older and thicker. Thus the young vertebra of *Cetorhinus* (Fig. 3) is tectospondylous. Outside the cyclospondylous "double cone" the intermedialia develop concentric arcs of calcification which together constitute outer cylinders interrupted only by the bases of the arches which penetrate to the double cone. As the vertebra grows older (Fig. 4) the intermedialia cease to produce arcs and produce wedges instead, in a word, the cyclospondylous condition becomes changed into an asterospondylous one.

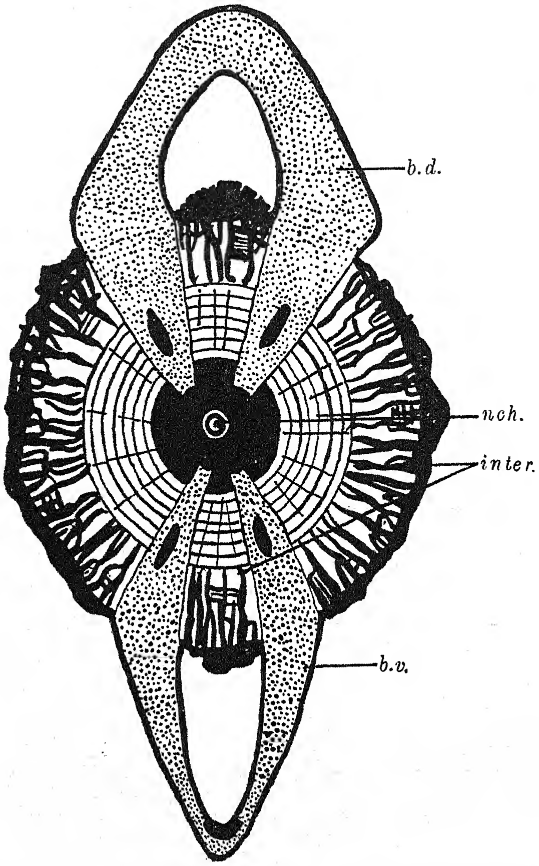

Fig. 4. Transverse section through a trunk vertebra of an older *Cetorhinus* in order to show combined tectospondyly and asterospondyly. The inner portions of the intermedialia show concentric arcs of calcification as in the young form, but in the outer portions radiating ridges of calcifications are seen which constitute asterospondyly. *b.d.* basidorsal; *b.v.* basiventral; *inter.* intermedialia; *nch.* remains of the notochord.

The common dog-fish (*Scyliorhinus* = *Scyllium*) has developed a very complex type of asterospondyly. The bases of the arches are enormously broadened and constitute a large part of the vertebra; they meet round the primitive double cone pushing the intermedialia outwards (Fig. 5). The intermedialia develop radiating wedges of calcification outside the cyclospondylous calcification where they flank the arch bases. In *Scyliorhinus marmoratus*, the primitive double cone itself develops four radiating wedges which project into the arch bases pushing the elastica externa before them, but this development does not apparently take place in *Scyliorhinus canicula*. The arches carry superficially abundant calcifications which remain isolated from one another like little scales (tesserae). It is thus possible to distinguish arch calcification from calcification of the intermedialia.

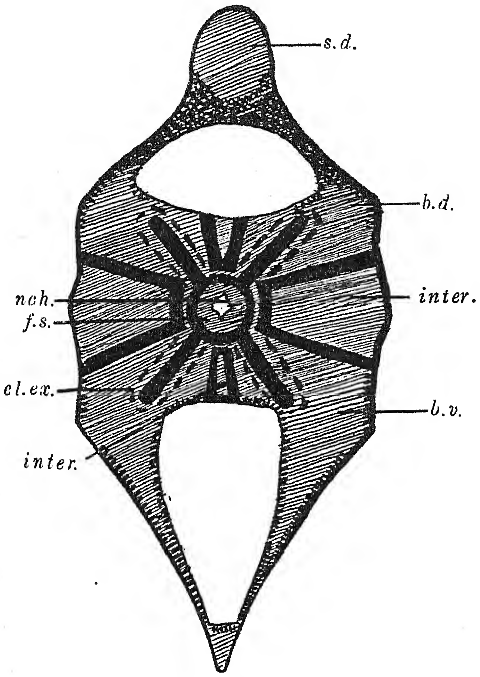

Fig. 5.

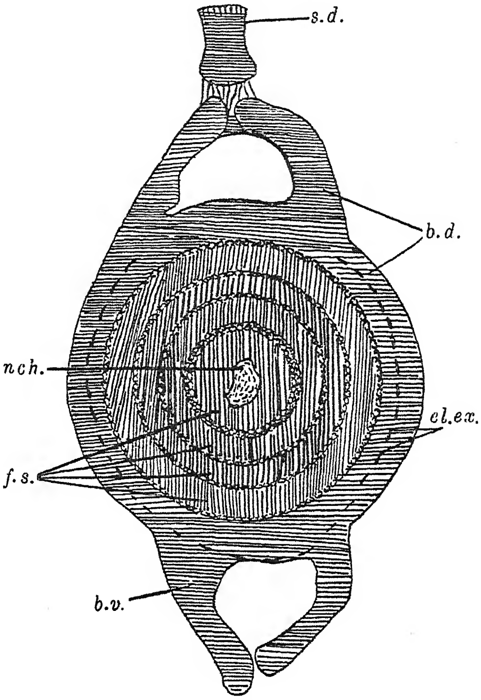

Fig. 6.

Fig. 5. Transverse section of the caudal vertebra of *Scyliorhinus* (*Scyllium*) in order to show the bulging of the primary centrum into the bases of the arches. Calcifications are coloured black. Those in the primary centrum and in the intermedialia are in the form of solid wedges (asterospondyly); those in the arches are in the form of scales (tesserae). *b.d.* basidorsal; *b.v.* basiventral; *el.ex.* elastica externa, the outermost layer of the primary sheath; *f.s.* fibrous sheath which becomes cartilage and constitutes the primary centrum; *inter.* intermedialia; *nch.* notochord; *s.d.* supradorsal.

Fig. 6. Transverse section of the caudal vertebra of a young *Squatina* in order to show a second form of "tectospondyly." The primary centrum or fibrous sheath has grown enormously and developed within itself concentric calcified rings. There are no intermedialia; the basidorsals and basiventrals meet each other outside the primary centrum. The boundary of this centrum is shown by the elastica externa which however is beginning to disappear. *b.d.* basidorsal; *b.v.* basiventral; *el.ex.* elastica externa; *f.s.* fibrous sheath = primary centrum with concentric rings of calcifications; *nch.* notochord; *s.d.* supradorsal.

In the rays (Batoidea) and the ray-like families of the true sharks (saw-fish Pristiuridae and angel-fish Rhinidae) the basidorsals and basiventrals meet round the primitive double cone and form an outer cylinder of cartilage. The primitive cone itself grows very much in diameter as the vertebra becomes older. *Squatina*, one of the Rhinidae, shows a type of tectospondyly of a totally different character from that described in the case of *Cetorhinus*. Outside the cyclospondylous cylinder a series of concentric cylinders is developed inside the elastica externa and therefore inside the primitive notochordal sheath itself. The intermedialia seem to be entirely absent (Fig. 6). The analysis of the vertebrae of rays is rendered difficult by the fact that the elastica externa is early absorbed, and it becomes almost impossible to say whether cartilage is derived from the chordal sheath, the bases of the arches or the intermedialia. Nevertheless putting all the evidence together it seems clear that the bulk of the vertebra is of chordal origin; that the intermedialia, if not entirely absent, are vestigial. The true rays, however, differ entirely from *Squatina* in that their calcifications consist of radiating wedges, an 8-rayed star being the characteristic shape seen in transverse section.

If this account be followed it will be seen that the term "chordacentrous" can only be used as a description of the vertebrae of the most primitive sharks and possibly of *Squatina*. The rays are arcocentrous and the majority of the sharks have vertebrae which are neither arcocentra nor chordacentra but are *perichordal centra*, and in this respect as we shall see they resemble the centra of other Vertebrata. In two respects, however, the vertebrae of sharks are at a level below those of the remainder of Vertebrata—first, no true bone is ever developed in them, and secondly, the neural arches do not articulate with one another and there are no zygapophyses.

2. Teleostei.

As has already been mentioned, Gadow in his researches on fish did not examine the vertebral column of any Teleost but confined his attention to the two "bony" Ganoids *Amia* and *Lepidosteus*. Amongst living Teleostei one of the most primitive types is represented by the herring, which has a long larval development including a stage resembling the Leptocephalus larva of the eel. Ramanujam's paper (1929), describing the development of the vertebral column, was based on a long series of stages beginning with embryos in which the alimentary canal had not been formed and ending with young herring in which all the adult features had been developed.

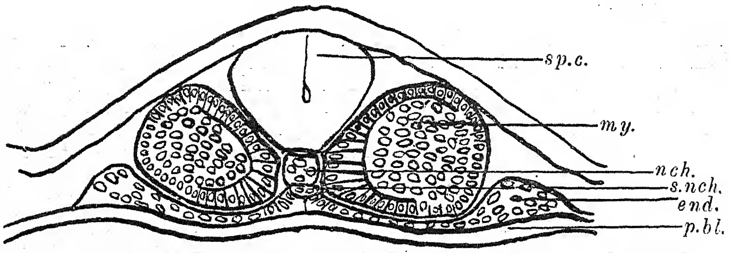

Fig. 7. Transverse section of a young embryo of the herring. The embryo is still spread out on the yolk and the endoderm has the form of a flat plate. *end.* endoderm; *my.* myotome; *nch.* notochord; *p.bl.* periblast; *s.nch.* subnotochordal rod; *sp.c.* spinal cord.

In his first stage (Fig. 7) the endoderm is represented by a plate of cells spread out flat on the yolk and separated from it only by the tenuous layer of cells known as the *periblast* (Fig 7 *p.bl.*) or yolk membrane. The notochord is a solid rod of cells and beneath it there is a still slenderer rod, the *subnotochord*. The myotomes are solid masses of cells, their outer layer consisting of columnar cells, whilst the core is composed of rounded cells; there is as yet no mesenchyme. In his next stage the flat plate of endoderm is folded up into a tube, which is the beginning of the alimentary canal, and mesenchyme is being budded off from the lower inner angle of the myotome (Fig. 8). This mesenchyme spreads downwards and upwards. It forms an investment for the notochord, the aorta and the spinal cord and evidently corresponds to the lower sclerotome of the Elasmobranch. In a later embryo (Fig. 9) mesenchyme is being liberated from the apical dorsal angle of the sclerotome; this is obviously the homologue of the dorsal sclerotome of the Elasmobranch, but Ramanujam found that it took no part in the formation of the basidorsal; the greater part of it migrates into the dorsal fin fold where it later gives rise to the cartilaginous fin rays, but it also gives rise to the dorsal elastic ligament which lies above the spinal cord between the apices of the basidorsals. A hollow outgrowth is developed from the apex of each myotome in *Amphioxus*, and, as Goldschmidt has shown, the gelatinous fin rays in the low dorsal fin of that fish develop from these outgrowths. It is therefore highly likely that the dorsal sclerotome will be found in the embryos of all fish,

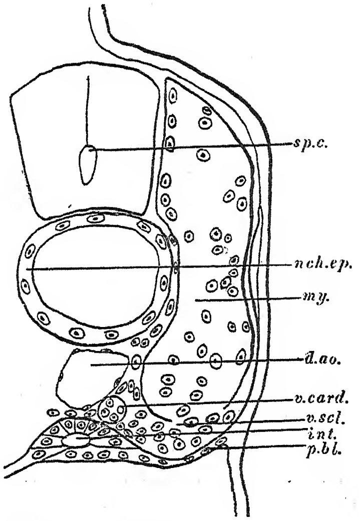

Fig. 8.

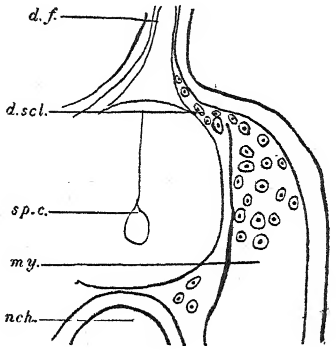

Fig. 9.

Fig. 8. Transverse section of an older embryo of the herring showing the formation of the lower sclerotome by budding from the myotome. The alimentary canal is beginning to be folded off from the yolk. *d.ao.* dorsal aorta; *int.* intestine; *my.* myotome; *nch.ep.* notochordal epithelium; *p.bl.* periblast; *sp.c.* spinal cord; *v.card.* cardinal vein; *v.scl.* ventral sclerotome.

Fig. 9. Transverse section of a still older embryo of the herring showing the formation of the dorsal sclerotome. *d.f.* rudiment of the dorsal fin; *d.scl.* dorsal sclerotome; *my.* myotome; *nch.* notochord; *sp.c.* spinal cord.

and that it is the rudiment not of any of the arcualia, but of the dorsal fin rays. The cells of the ventral sclerotome form a series of half-hoops flanking the notochord, each hoop corresponding to a myotome. These half-hoops evidently correspond to the intermedialia of the sharks. The half-hoops of the same side are connected to their successors by two longitudinal ridges of cells, one dorsal and one ventral.

Shortly afterwards the embryo hatches and becomes a larva (Fig. 10). The half-hoops of mesenchyme cells now become connected to their successors and form a continuous perichordal sheath and in addition clusters of sclerotomic cells extend into the myocommata; these are the rudiments of the basidorsals. Beneath the perichordal sheath a true chordal sheath has been developed. This is a thick fibrous structure with dorsal and ventral thickenings which give rise to the *inferior dorsal ligament* and to the *ventral ligament* respectively. The cells of the notochord tend to move outwards and become aggregated beneath the chordal sheath forming the so-called notochordal epithelium (Fig. 10 *nch.ep.*). Beneath the chordal sheath outside the actual notochordal cells there is a gelatinous layer which appears in sections as an empty space. Thickenings of mesenchyme cells, already becoming pro-cartilaginous, which are formed beneath the extreme posterior end of the notochord, prefigure the tail skeleton. When the larva has attained the length of 3 cm. the first formation of bone takes place, and as a consequence the formation of the *centra* (Fig. 11). These centra are formed by the direct ossification of the chordal sheath and are therefore *chorda-centra*. Ramanujam's evidence is that this ossification is brought about by the migration through the elastica externa of minute needle-shaped mesenchyme cells from the bases of the arches. These centra are formed generally opposite the break between two myotomes, but their position varies, and in some places, as in the anterior end of the larva, they are opposite the anterior end of the myotome. Gadow in discussing the Teleostei comments on the varying position of the arches in the dried skeleton in different genera of Teleostei and suggests that the group may be polyphyletic. Ramanujam's evidence shows that the arches are always constant in position since they are situated in the myocommata; what varies is the position of the centrum, and this can change as we pass from front to back in the same specimen.

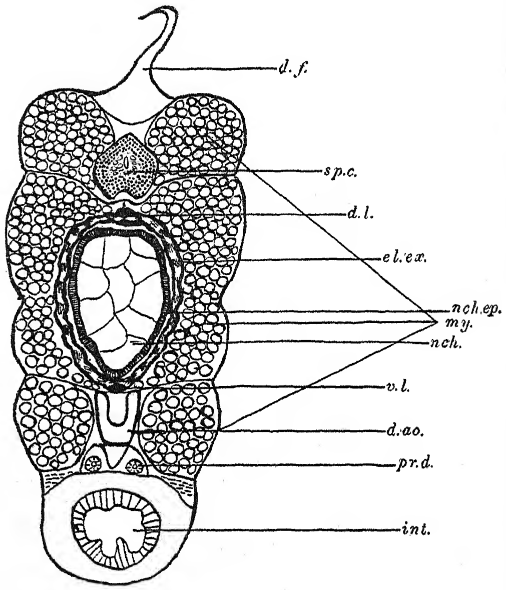

Fig. 10. Transverse section of a herring larva 1·6 cm. long showing the formation of the first notochordal sheath. *d.ao.* dorsal aorta; *d.f.* rudiment of the dorsal fin; *d.l.* inferior dorsal ligament, a thickening of the sheath; *el.ex.* elastica externa, the outermost layer of the sheath; *int.* intestine; *my.* myotome; *nch.* notochord; *nch.ep.* notochordal epithelium; *pr.d.* pronephric duct; *sp.c.* spinal cord; *v.l.* ventral longitudinal ligament, also a thickening of the chordal sheath.

When the larva has reached the length of 3 cm. the outer perichordal sheath begins to ossify, and thus a series of outer bony cylinders, which we may term *perichordal centra*, are formed (Fig. 12). The notochordal tissue intervening between the chorda-centra projects as a series of gelatinous pads which are ultimately transformed into the intervertebral ligaments. The basidorsals which have become cartilaginous sit in cups in the outer or perichordal centra. Basiventrals have been formed by the segmentation of the ventral longitudinal ridge of mesenchyme mentioned above; these are similarly attached to the perichordal centra. A little later the outer layer of the tips of the basidorsals begins to ossify, but their bases

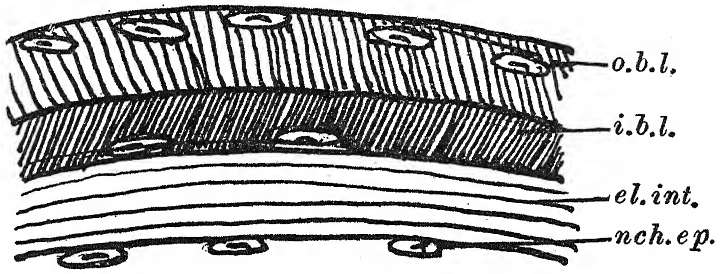

Fig. 11. Portion of a transverse section through the notochord of a herring larva 3 cm. long. *el.int.* elastica interna. The innermost layer of the notochordal sheath; *i.b.l.* inner bony layer of the centrum formed from the notochordal sheath (chiefly the elastica externa); *nch.ep.* notochordal epithelium; *o.b.l.* outer bony layer of the centrum formed from the perichordal mesoderm layer.

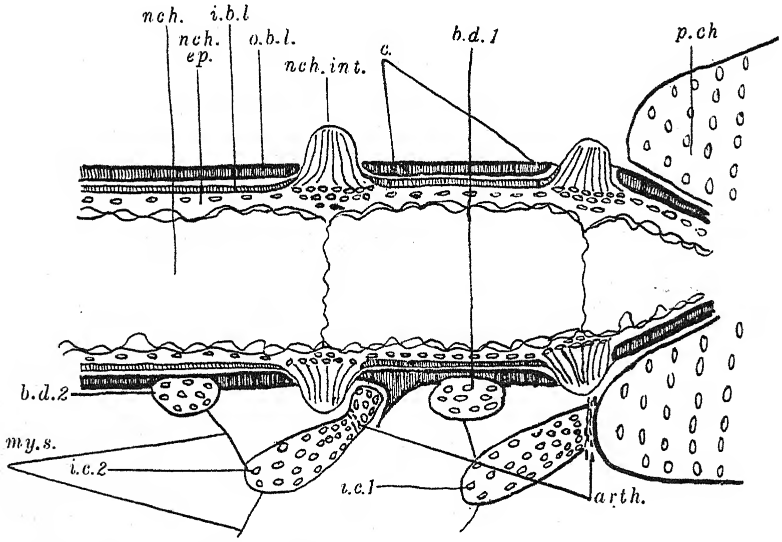

Fig. 12. Frontal section through the notochord and back of the skull of a herring larva 4·5 cm. long. *arth.* ingrowing connective tissue forming a joint; *b.d.* 1, first basidorsal; *b.d.* 2, second basidorsal; *c.* centrum; *i.b.l.* inner bony layer of centrum; *i.c.* 1, first intercalary; *i.c.* 2, second intercalary; *my.s.* myoseptum; *nch.* notochord; *nch.ep.* notochordal epithelium; *nch.int.* intervertebral ring of the notochord; *o.b.l.* outer bony layer of the centrum; *p.ch.* parachordal cartilage of the skull.

remain soft cartilage. Ramanujam was not able to detect the formation of dorsal and ventral intercalaries throughout the vertebral column. Only two pairs of dorsal intercalaries were found, one pair lying between the occipital cartilage and the first pair of basidorsals and one pair between the first and second pair of basidorsals.

About this time between the ossifying apices of the basidorsals the supradorsal cartilages are formed. These are paired cartilages adhering to the inner sides of the basidorsals (Fig. 13) and supporting the dorsal ligament. Ramanujam regards them as equivalent to the supradorsals of Elasmobranchs, but this is not quite clear and further investigation is desirable. Another point on which Ramanujam is unsatisfactory is his account of the origin of the zygapophyses. The neural arches of the herring, like those of all Vertebrata higher in the scale, articulate with one another by projecting facets known as zygapophyses. Ramanujam describes these as vertical outgrowths of the "outer bony ring," *i.e.* the perichordal centra "formed directly from connective tissue," and later he states that the basidorsal of each segment is connected with its zygapophysis by "a bony ridge." This whole matter requires re-examination and re-statement. The strong probability is that Ramanujam is mistaken as to the origin of the zygapophyses. If, as seems likely, they

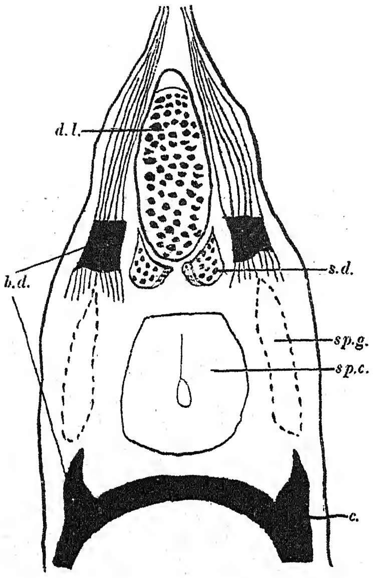

Fig. 13.

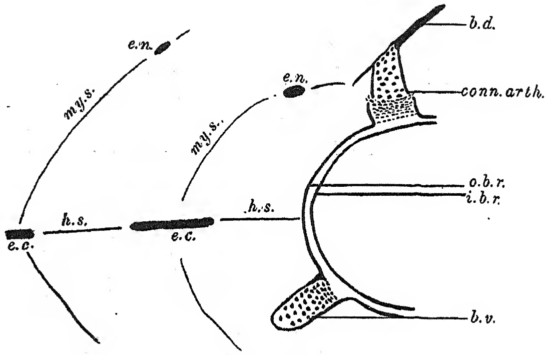

Fig. 14.

Fig. 13. Transverse section through the dorsal portion of a herring larva 6·2 cm. long. *b.d.* bony basidorsal; *c.* centrum; *d.l.* superior dorsal ligament above the spinal cord; *s.d.* supradorsal cartilages; *sp.c.* spinal cord; *sp.g.* spinal ganglia lying in gaps in the basidorsal.

Fig. 14. Transverse section through a herring larva 5·6 cm. long. *b.d.* basidorsal bony but with a core of cartilage cells; *b.v.* basiventral bony but with a core of cartilage cells; *conn.arth.* connective tissue cells growing in at the base of the neural arch so as to form a joint; *e.c.* epicentral bone; *e.n.* epineural bone; *h.s.* horizontal septum; *i.b.r.* inner bony ring of centrum; *my.s.* myoseptum; *o.b.r.* outer bony ring of the centrum.

are formed as extensions of the supradorsals then these pieces will correspond to the so-called "dorsal interdorsals" of the higher forms.

The rib as in the Elasmobranch is primarily a prolongation of the cartilaginous basiventral; but unlike the rib of the Elasmobranch it does not project outwards into the horizontal septum which divides the myotome into dorsal and ventral portions, but passes below the myotome along the dorsal wall of the peritoneal cavity. But from the apex of the ventralia a band of connective tissue extends outwards into the horizontal septum. This band soon ossifies as the *epicentral* bone—a structure peculiar to the herring and some other primitive Teleostei and in all probability corresponding to the Elasmobranch rib (Fig. 14). When the true rib is cut off from the basiventral, its inner end unites with the inner end of the epicentral bone. From the outer side of the basidorsal, a belt of ossifying connective tissue extends outwards and downwards in the myocomma and forms the *epineural bone*.

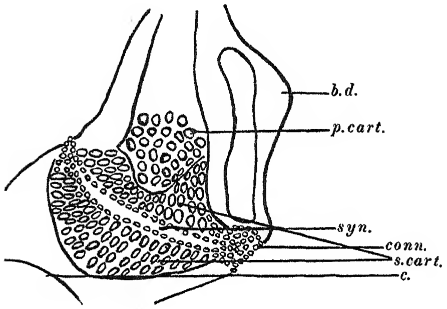

Fig. 15.

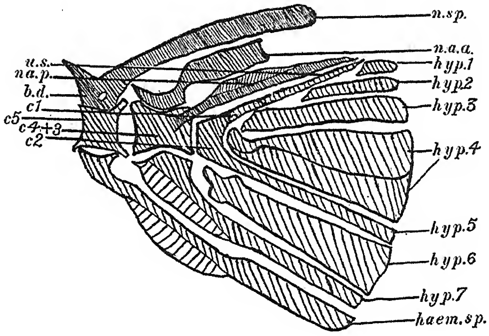

Fig. 16.

Fig. 15. Transverse section through the base of one of the neural arches of a herring larva 6·2 cm. long. *b.d.* basidorsal; *c.* centrum; *conn.* ingrowing connective tissue; *p.cart.* primary cartilage of the basidorsal; *s.cart.* secondary joint-cartilage; *syn.* synovial cavity.

Fig. 16. Preparation of the caudal skeleton of a herring larva about 10 cm. long stained with alizarine. *b.d.* basidorsal of the last normal centrum; c. 1, c. 2, c. 4 + 3, c. 5, the centra which enter into the composition of the tail region. These are reckoned from behind forwards, so that c. 1 is the last of these centra, c. 2 is the penultimate centrum and so on; c. 1 and c. 2 are fused with the urostyle; c. 3 and c. 4 form a compound centrum and c. 5 is the last normal centrum; *haem.sp.* the haemal spine attached to the last normal centrum; *hyp.* 1–7 are the seven hypural bones, these are modified and enlarged haemal spines and are reckoned from behind forwards so that *hyp.* 1 is the most posterior; *n.a.a.* and *n.a.p.* are the anterior and posterior neural arches attached to the compound centrum c. 4 + 3; *u.s.* is the urostyle, the bony sheath of the extreme posterior end of the notochord.

At first the bases of both basidorsal and basiventral consist of soft round-celled cartilage continuous with the cartilage of the "perichordal centra." But a little later joints between the ribs and basal "stumps" are formed and similar joints arise between the bases of the neural arches and the perichordal centra. The joints are formed by the ingrowth of a ring of external cells which cuts the base of the arch into two pieces separated by a synovial cavity (Fig. 15). The invading cells arrange themselves into two layers flanking this cavity and subsequently themselves become transformed into hyaline cartilage.

The herring, like all Teleostei, possesses a complicated caudal skeleton which, as already explained, is laid down as procartilage in the very young larva. Its early appearance and elaborate development correspond with the importance of the tail as a locomotive organ. The adult condition is attained when the fish is 10 cm. long (Fig. 16). The notochord at its posterior end is bent upwards and the caudal fin is really a ventral flap of skin. The ossified end of the notochord is termed the *urostyle*. In front of this, attached to it by suture, is a slender bony cylinder which Ramanujam calls centrum 1. This is similarly attached in front to an obviously normal centrum which is termed centrum 2. In front of this and separated from it by ligament is a cylinder representing two fused centra; this structure is termed centrum 3 + 4. In front of this is the last normal centrum of the vertebral column which is numbered centrum 5; it carries a long backwardly projecting neural spine. The compound centrum 3 + 4 carries two shorter neural spines—these three constitute what Tate Regan calls the "uroneurals." The fin itself is supported by a series of broad flattened bones, "hypurals," which represent haemal spines fused with fin rays. Of these the most anterior, numbered 7, is borne by centra 3 + 4. Behind this is hypural 6, belonging to the same centrum but detached from it and suspended in connective tissue. Hypural 5 is attached to centrum 2 and hypurals 4, 3, 2 and 1 float freely in the connective tissue beneath the cylinder composed of the urostyle and centrum 1. The urostyle must therefore represent at least three centra.

If we now sum up the main conclusions of Ramanujam's paper we find them to be as follows:

1. There is a dorsal sclerotome as well as a ventral sclerotome, but the ventral sclerotome gives rise to the membrana reuniens, whilst the dorsal sclerotome gives rise to the dorsal ligament and the dorsal fin rays.

2. The centra of the herring are compound structures; there is an inner cylinder which is a true chorda-centrum and there is an outer cylinder formed from the perichordal sheath and corresponding to the intermedialia of the Elasmobranch; these two cylinders are easily distinguishable in the adult vertebra.

3. The epicentral bone grows out from the basiventral cartilage and corresponds to the Elasmobranch rib; the true rib is directed ventrally and it corresponds to the rib of the higher Vertebrata.

4. The cartilaginous basidorsals and basiventrals are at first continuous with the perichordal cartilage. The basidorsals become separated from this cartilage by secondary joints formed by ingrowing connective tissue; the inner portions of the basiventrals adhere to the centrum and are known as "parapophyses," their outer portions become separated from the inner portions by secondary joints and are ribs.

III. AMPHIBIA.

1. Urodela.

We may now consider the development of the vertebral column of Amphibia and begin with Mookerjee's paper (1930 *a*) on *Triton*. Mookerjee's material consisted of an unbroken series of stages from the formation of the germinal layers in the young embryo up to the complete metamorphosis of the larva. He had also a certain number of post-larval stages, although these are exceedingly difficult to obtain. Contrary to the popular opinion the newt is not an aquatic animal; it merely enters the water in the spring to breed. When the larva has completed its metamorphosis it emerges from the water and does not return to it for four years, until in fact it has become sexually ripe. Young immature newts are found amongst the stems of heather, under damp stones and in similar hiding places and are by no means easy to find.

In the embryo of the newt the notochord acquires the same two sheaths as in the herring, viz. an inner chordal sheath composed of a thin elastica externa outside and a fibrous elastica interna inside, and an outer perichordal sheath consisting of mesenchyme cells. These mesenchyme cells are formed from a series of ventral sclerotomes on the inner borders of the myotomes; dorsal sclerotomes do not exist. These cells at first constitute a series of perichordal rings round the notochord, each ring being situated opposite the middle of a myotome; but whilst the main aggregations remain here, scattered cells extend from them along the notochord, so that a continuous perichordal tube is formed. The true chordal sheath is never penetrated by cells and remains membranous. Not all of the sclerotome is used up in forming the perichordal tube; scattered mesenchyme cells forming part of the membrana reuniens extend round the spinal cord. A little later marked aggregations of cells on each side of the dorsal aspect of the notochord appear; these aggregations are opposite the hinder end of a myotome—or, to speak more correctly, opposite a myocomma—and are the rudiments of the basidorsals. When these basidorsals are converted into cartilage this cartilage remains sharply distinguishable by the size and disposition of its contained cells from the cartilage of the perichordal sheath. Opposite the middle of the myotome is found the spinal ganglion. The vertebral centrum is later formed by an ossification of a portion of the perichordal tube lying opposite the mycomma—just as in the herring. The front part of the centrum carries the basidorsals which correspond to the hinder part of the myotomal segment in front, whilst above the hinder part of the centrum lies the spinal ganglion belonging to the front part of the myotomic segment behind. This alternation of vertebrae and myotomic segments first described by Remak (1851) is what is termed the "resegmentation of the vertebral column."

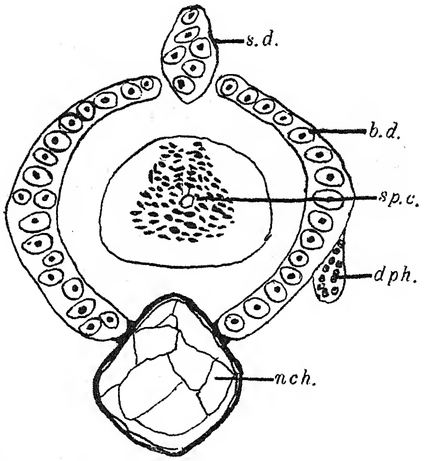

Fig. 17. Transverse section of a larva of a newt 20 mm. long. *b.d.* cartilaginous basidorsal; *dph.* rudiment of the diapophysis (= rib-bearer); *nch.* notochord; *s.d.* supradorsal cartilage; *sp.c.* spinal cord.

In the trunk region no basiventral aggregations of cells or basiventral cartilages are formed, but after the embryo has hatched and become a larva, an aggregation of cells is formed in the myocomma some distance from the vertebral column which is the rudiment of the rib. In the tail region, however, aggregations of cells giving rise to cartilaginous basiventralia are formed on the ventral aspect of the notochord. As the larva grows older the basidorsals grow upwards and enclose the spinal cord, but the enclosure is not complete; they are separated at their apices by an unpaired series of cartilages, the *supradorsals* (Fig. 17). Each supradorsal gives rise at its hinder edge to two bars of cartilage which grow backwards until they reach the next arch behind. A joint is formed by the ingrowth of a ring of connective tissue cells and the bar is cut into two sections; the longer section constitutes the *postzygapophysis* of the vertebra in front and the smaller the *prezygapophysis* of the vertebra behind (Fig. 22). The sides of the basidorsal develop forked outgrowths termed *diapophyses* or "rib-bearers" (*dph.* Fig. 17), to which the rib ultimately becomes attached.

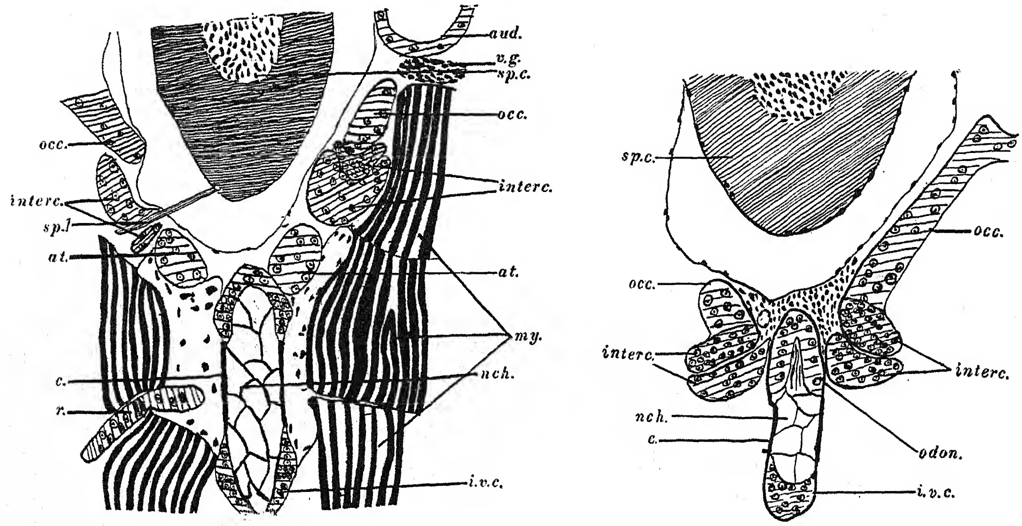

Fig. 18. Fig. 19.

Figs. 18, 19. Two frontal sections through the anterior part of a larval newt 25 mm. long. Fig. 18 represents the more dorsal section. The dorsal section shows the myotomes, the auditory capsule, the vagus ganglion and the first spinal nerve; *at.* so-called "atlas arch" attached to the first vertebra; *aud.* auditory capsule; *c.* the first centrum; *interc.* the two portions of the intercalary arch which intervenes between the occipital cartilage and the vertebral column; *i.v.c.* intervertebral cartilage; *occ.* occipital cartilage; *my.* myotomes; *nch.* notochord; *odon.* the so-called odontoid process of the first vertebra; *r.* rib growing in towards the centrum; *sp.c.* spinal cord; *sp.*1. first spinal nerve; *v.g.* vagus ganglion.

The connection of the vertebral column with the skull was carefully examined by Mookerjee and presents extremely interesting features. It is represented in Figs. 18 and 19. If we bear in mind that a myotomic segment carries a spinal ganglion in front and a cartilaginous arch behind we see that the region of the skull behind the auditory capsule corresponds to one myotomic segment and one only. The ganglion is the vagus ganglion and the arch the occipital arch. Between the skull, however, and the first completely formed neural arch a rudimentary "intercalary arch" is interposed. This arch becomes cut into two pieces by ingrowing connective tissue cells. The front part adheres to the occipital cartilage of the skull and forms on each side the *occipital condyle*; the hinder part adheres to the front aspect of the first complete neural arch (the so-called atlas arch) and forms the *cup* with which the condyle articulates. Between the cup and the condyle the first spinal nerve (the nervus sub-occipitalis) is given off (Fig. 18). There is no centrum corresponding to this intercalary arch; but the anterior end of the notochord is ensheathed in a thick cartilage which corresponds to one of the intervertebral cartilages—and the ingrowth of connective tissue cells, which cuts each intercalary cartilage into two, slants forwards and inwards and unites with its fellow so as to cut this intervertebral cartilage, the so-called *odontoid process* (Fig. 19, *odon.*) from the occipital cartilage of the skull. It is necessary to insist that the so-called atlas arch and odontoid process in no way correspond to the similarly named structures in the Amniota.

We must now return to a consideration of the vertebral column. In the young larva, as we have seen, the notochord is enveloped in a continuous fibro-cartilaginous tube, which is developed into a thick disc opposite the middle of each myotome. The basidorsals (and in the tail the basiventrals) are attached to the thin intermyotomic portions of this tube.

In the late larva (about 25 mm. in length) ossification sets in, and the thin portions of the tube become converted into bony centra, whilst the thick portions remain as intervertebral cartilaginous discs. Certain genera of newts (*Amphiuma* and *Menobranchus*) are described as amphicoelous. This is an extremely misleading statement. True amphicoelous vertebrae such as those of the herring are separated by expansions of the notochord, but the so-called amphicoelous vertebrae of these Amphibia are connected by thick pads of cartilage which obliterate the notochord altogether. The notochordal cells in the middle of each vertebral region become actually converted into a cartilaginous disc, the *intravertebral cartilage*, the sole instance of such a transformation of notochordal tissue which has ever been recorded.

In the ossification of the arcualia, the basidorsals receive an outer covering of bone, but the inner perichondrium and the whole of the cartilaginous cells degenerate, so that the bony arch is much thinner than the cartilaginous one (Fig. 21). The process of ossification once started extends forwards and backwards into the fibrous membrana reuniens connecting successive neural arches and forms what Mookerjee calls "anterior" and "posterior" connective tissue arches. The supradorsal becomes ensheathed by bone, but this covers only a small part of the spinal cord, and the ossification extends in front of it so as to form a "connective tissue neural roof." In virtue of these processes the vertebra becomes long from before and behind and is separated from its successor by a very small region of unossified fibrous tissue. Where the connective tissue arch meets the connective tissue roof a prominent projecting shelf is formed which can be recognised in the adult vertebra (Fig. 25).

As metamorphosis is approached, the supradorsal cartilage and diapophysis become ossified and the rib grows inwards and becomes attached to the forked diapophysis. It was natural to compare this fork to the forked attachment of the Amniote rib to its centrum. But between the two forks of the Amniote rib runs the vertebral artery, whilst the vertebral artery of the Urodele is ventral to both of them (Figs. 23 and 24). If the Urodele rib corresponds in the way suggested to the Amniote rib, it is necessary to assume that in evolution the lower fork of the

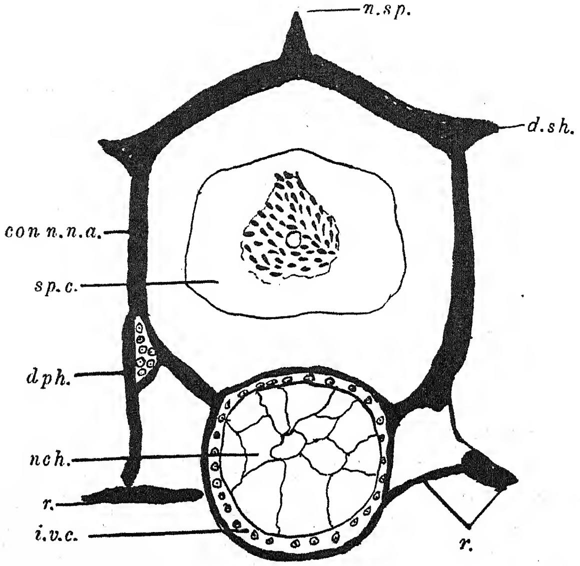
n.sp.
d.sh.
con n.n.a.
sp.c.
dph.
nch.
r.
i.v.c.
r.

Fig. 20.

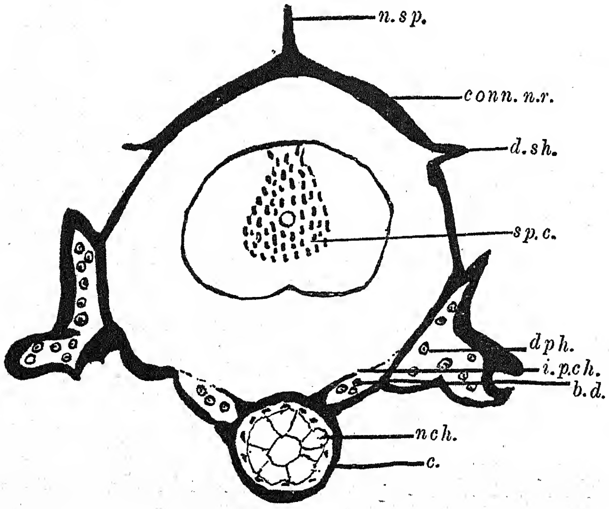
n.sp.
conn. n.r.
d.sh.
sp.c.
dph.
i.p.ch.
b.d.
nch.
c.

Fig. 21.

Urodele rib moved downwards across the vertebral artery, and this transformation is actually assumed by Goodrich (1930) in his recent text-book on Vertebrata, a transformation which it seems impossible to the present writer to conceive.

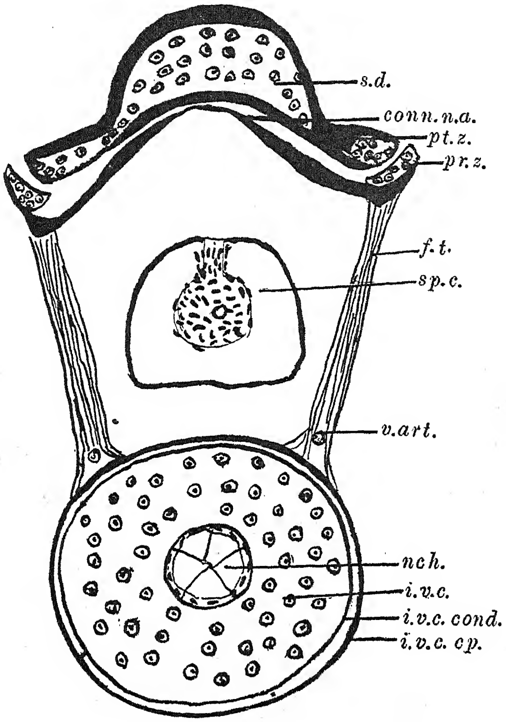

Fig. 22.

Figs. 20, 21, 22. Three transverse sections through the vertebral column of a newt larva 35 mm. long. Fig. 20 goes through the front end of a vertebra, Fig. 21 goes through the middle of a vertebra and Fig. 22 goes through the hind end of a vertebra. Ossified tissue is represented by thick black lines. In the front section the spinal cord is covered by ossified connective tissue: the so-called connective tissue arch. The diapophysis (= rib-bearer) has grown downwards and has nearly reached the ingrowing rib. The notochord is surrounded by the anterior intervertebral disc. In the middle section the cartilaginous rib-bearer surrounded by its bony cover is seen to be bifurcated. The base of the cartilaginous basi-dorsal is seen to be covered inside and out by a perichordal layer which is being converted into bone—but the inner perichordal layer is degenerating. In the hinder section the posterior cartilaginous inter-vertebral disc is cut: this constitutes the condyle of the vertebra: outside this is the cup of the next vertebra. The supradorsal cartilage and its prolongations the posterior zygapophyses are cut. The sides of the spinal cord in this region are unprotected except by fibrous tissue. *b.d.* cartilaginous basi-dorsal; *c.* centrum; *conn.n.a.* connective tissue neural arch; *conn.n.r.* connective tissue neural roof; *d.sh.* dorsal shelf of this; *dph.* cartilaginous rib-bearer (= diapophysis); *f.t.* fibrous tissue; *i.p.ch.* inner degenerating perichondral layer of the basidorsal; *i.v.c.* intervertebral cartilage; *nch.* notochord; *i.v.c.cond.* condylar portion of the intervertebral cartilage; *i.v.c.cp.* cup portion of the same; *pr.z.* prezygapophysis; *pt.z.* postzygapophysis; *s.d.* supradorsal; *sp.c.* spinal cord; *v.art.* vertebral artery.

The riddle was solved by Gray (1930). He showed that the rib in its inward growth towards the centrum, after joining both forks of the diapophysis, continued its growth, and passing *beneath* the vertebral artery formed a third attachment to the centrum, which obviously corresponds to the head of the Amniote rib. When metamorphosis is complete there are still large remains of the notochord within the centrum, and the cartilaginous intravertebral disc is completely divided

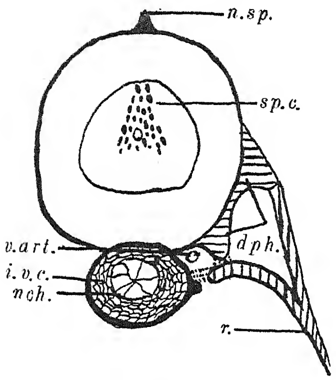

Fig. 23.

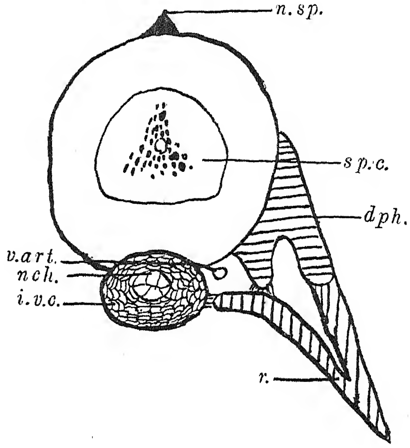

Fig. 24.

Figs. 23, 24. Two stages in the development of the attachments of the rib to the vertebra. Fig. 23 is the younger. *dph.* forked diapophysis; *i.v.c.* intervertebral cartilage; *nch.* notochord; *n.sp.* neural spine; *r.* rib; *sp.c.* spinal cord; *v.art.* vertebral artery.

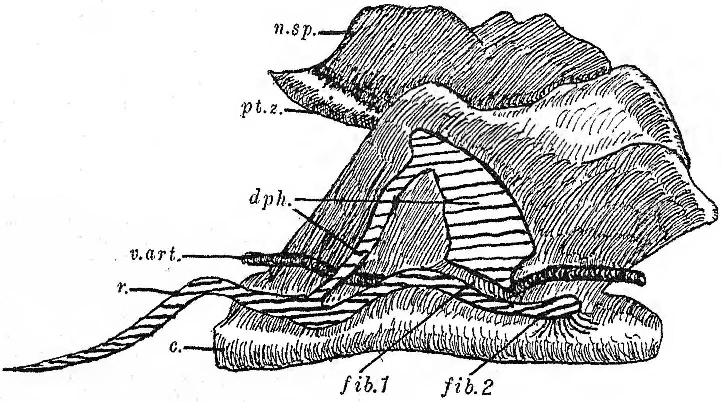

Fig. 25. Side view of the vertebra of a metamorphosed newt showing the attachments of the rib. *c.* centrum; *dph.* forked diapophysis; *fib.* 1, fibrous attachment of the rib to the diapophysis *above* the vertebral artery; *fib.* 2, fibrous attachment of the rib to the centrum *below* the vertebral artery; *n.sp.* neural spine; *pt.z.* postzygapophysis; *r.* rib; *v.art.* vertebral artery.

into an anterior ball attached to the vertebra in front and a posterior cup attached to the vertebra behind, so that an opisthocoelous joint is formed (Fig. 22). In the tail region, as we have seen, distinct haemal arches are formed. Between the apices of the haemal arches median, interventral bodies are formed and when ossification sets in, below the notochord as above it, "connective tissue arches" are formed.

2. ANURA.

In his investigation of the development of the vertebral column of Anura, Mookerjee (1930 *b*) had at his disposal a very complete series of the embryos and tadpoles of *Rana temporaria* and of the Indian toad (*Bufo melanostictus*). He had also a few stages of the development of the European fire-toad (*Bombinator igneus*), and rather more stages of the development of the African water toad (*Xenopus laevis*).

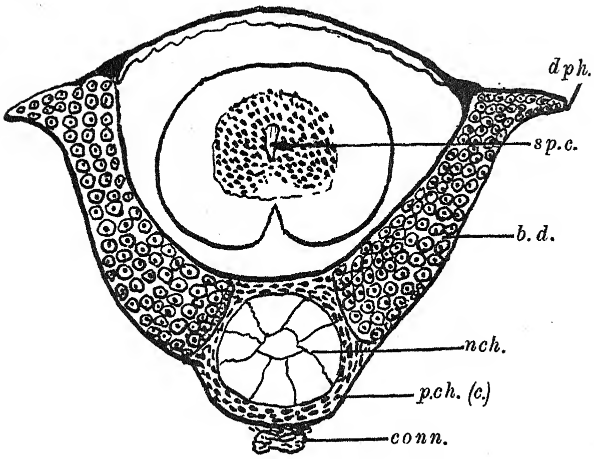

Fig. 26. Transverse section through the middle of the vertebral region of a tadpole of *Rana temporaria* 25 mm. long. *b.d.* basidorsal cartilage; *conn.* ventral mass of connective tissue representing the basiventral cartilage; *dph.* diapophysis; *nch.* notochord; *p.ch.*(*c.*) the region of the perichordal tube which is later converted into the centrum.

The post-larval stages of *Rana* are almost as difficult to obtain as the post-larval stages of the newt, nevertheless by assiduous collecting he obtained a good store of these. In its general outlines the development of the vertebral column in Anura follows the same lines as in Urodela. The main differences are caused by the absence of obvious ribs in Anura and the absence of a tail.

The formation of the sheaths of the notochord, of the perichordal rings of the fibro-cartilaginous perichordal tube and of the dorsolateral accumulations of cells which are the rudiments of the basidorsals take place in exactly the same way as in the newt. But no supradorsal is formed (Fig. 26) and later when the diapophysis is formed this is not forked. The basidorsals at first extend only half way up the spinal cord and are connected with each other by a membranous roof, but later they grow so as to meet each other and they give off from near their point of meeting cartilaginous bars which grow backwards and become divided into pre- and post-zygapophyses and which represent the supradorsal of the Urodele with its two processes.

Beneath the notochord outside the perichordal sheath in the mid-ventral line, there is a mass of connective tissue which probably represents the missing basiventralia (Fig. 26, *conn.*). Behind the union of the aortic arches, in the centre of this mass, there is differentiated a cylindrical rod of cartilage, the *hypochord*, which later enters into the composition of the urostyle (Fig. 27, *hyp.*).

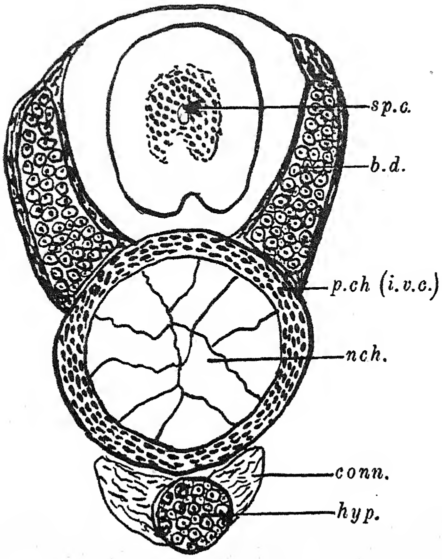

Fig. 27.

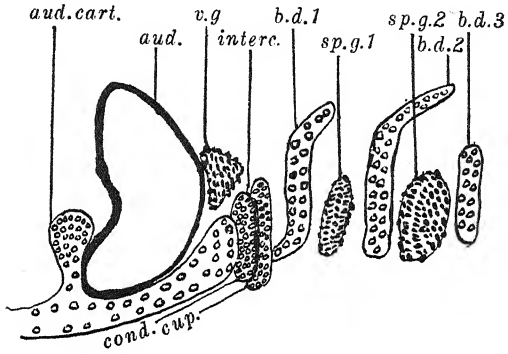

Fig. 28.

Fig. 27. Transverse section through the urostyle region of a metamorphosing tadpole of *Rana temporaria* 32 mm. long. *b.d.* basidorsal cartilage; *conn.* masses of connective tissue representing the basiventrals; *hyp.* hypochordal cartilage; *nch.* notochord; *p.ch.* (*i.v.c.*) intervertebral region of the perichordal cartilaginous tube; *sp.c.* spinal cord.

Fig. 28. Sagittal section through the occipital region and vertebral column of a young toad (*Bufo melanostictus*) just after metamorphosis. *aud.* auditory capsule; *aud.cart.* auditory cartilage; *b.d.* 1, *b.d.* 2, *b.d.* 3, the first, second and third basidorsals; *cond.* occipital condyle; *cup.* socket for the cup on the first vertebra; *interc.* intercalary arch dividing into condyle and cup; *sp.g.* 1, *sp.g.* 2, first and second spinal ganglion; *v.g.* vagus ganglion.

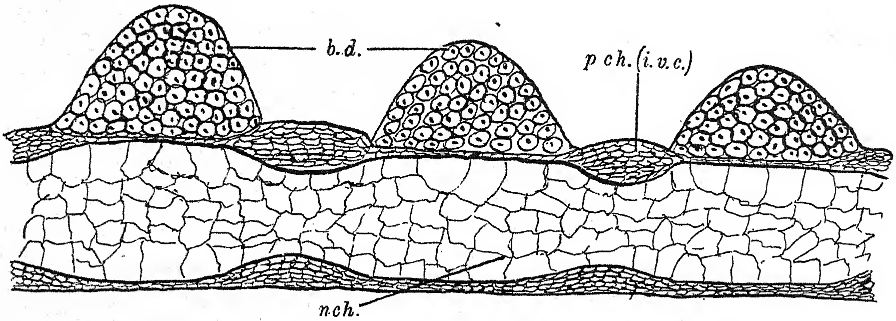

Fig. 29. Oblique frontal section through the trunk of a tadpole of *Rana temporaria* 22 mm. long. *b.d.* basidorsals; *nch.* notochord; *pch.* (*i.v.c.*) intervertebral portion of the cartilaginous perichordal tube.

The connection of the vertebral column to the skull is effected in the same way as in Urodela. An intercalated arch is interposed between the skull and the first vertebra. This arch is divided into occipital condyle and atlas cup on each side, and between these divisions in the toad, but not in the frog, the sub-occipitalis nerve passes out (Fig. 28). The intervertebral cartilage investing the front end of the notochord makes a flat suture with the occipital cartilage, so that there is no "odontoid process." During the whole of the larval life until the tail begins to shorten and metamorphosis approaches, no important changes take place in the vertebral column. The notochord is invested in a continuous fibro-cartilaginous tube slightly thickened in the intervertebral regions and very thin in the vertebral regions beneath the basidorsals (Fig. 29), and as in the Urodele the cartilage of the tube is clearly distinguishable from that forming the basidorsals. But as metamorphosis approaches, after the basidorsals have become cartilaginous the 9th and 10th neural arches fuse with one another, and then become separated by ingrowing connective tissue so as to form a cup and ball articulation on each side, the ball being formed by the 9th arch and the cup by the 10th arch, the hypochord be-

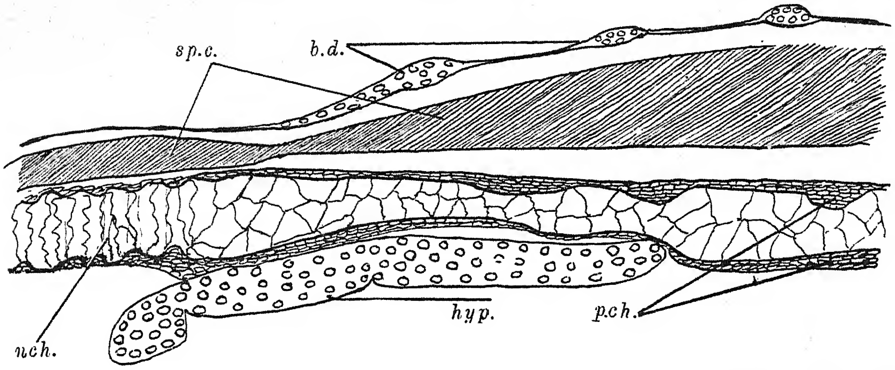

Fig. 30. Sagittal section through the hinder portion of a metamorphosing tadpole of *Rana temporaria* with shrinking tail, 20 mm. long. *b.d.* basidorsals—the hinder one of these will form the dorsal ridge on the urostyle; *hyp.* hypochordal cartilage forming the ventral portion of the urostyle; *nch.* shrivelling notochord; *pch.* perichordal cartilaginous tube showing intervertebral swellings; *sp.c.* spinal cord.

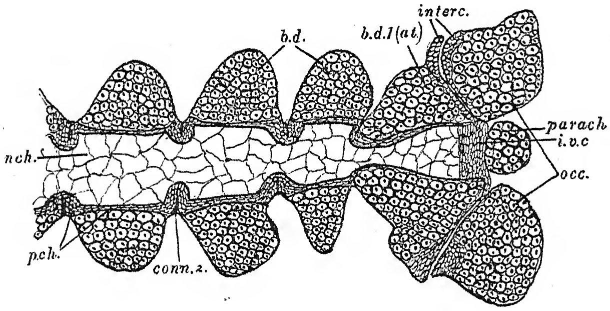

Fig. 31. Front section of the hinder part of the skull and of the vertebrae of a young toad (*Bufo melanostictus*) just after metamorphosis. *b.d.* 1 (*at.*) first basidorsal forming the so-called atlas arch; *conn. z.* invading sheet of connective tissue which will divide the intervertebral cartilage into two parts; *interc.* intercalary cartilage already divided into condyle and cup; *i.v.c.* intervertebral cartilage between the skull and the vertebral column; *nch.* notochord; *occ.* occipital cartilage; *pch.* perichordal tube.

comes greatly developed and presses upwards on the notochord which decreases in diameter. Behind the hypochord in the genuine tail region the notochord shrinks longitudinally and the septa (remains of the notochordal cell walls) come closer together. Eventually the 10th arch and the 11th arch, which is fused with it, unite with the hypochord, squeezing out the notochord altogether and so the urostyle is constituted. The neural arches form dorsal ridges on its surface and the whole is converted into bone (Fig. 30).

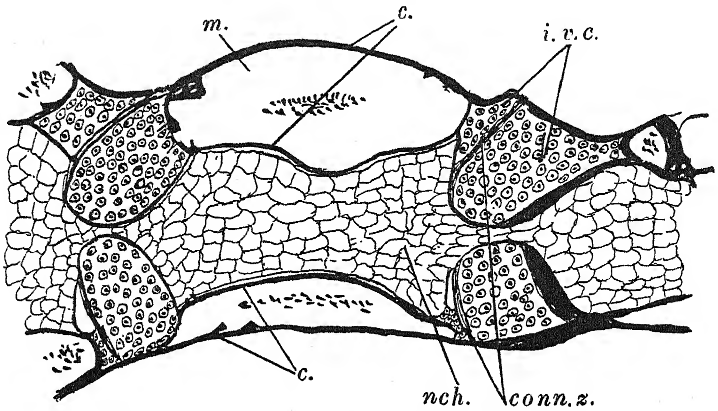

Fig. 32. Frontal section through a trunk vertebra of a young *Rana temporaria* about 2 years old. *c.* centrum ossified and hollowed out by marrow spaces; *conn. z.* connective tissue growing into and dividing the intervertebral cartilage into two; *i.v.c.* intervertebral cartilage; *m.* marrow space; *nch.* notochord.

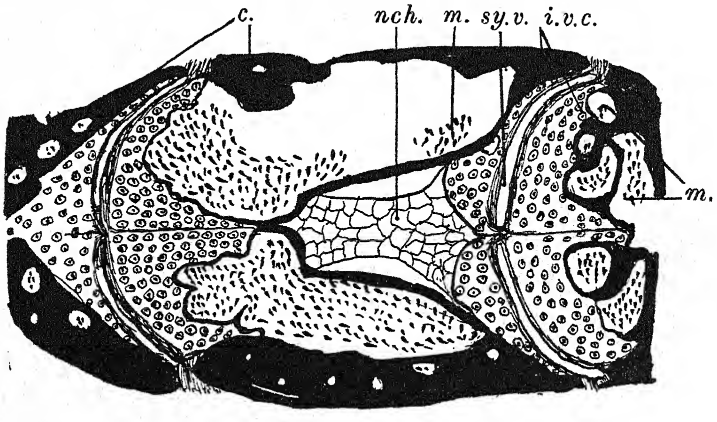

Fig. 33. Frontal section through a trunk vertebra of a young *Rana temporaria* about 3 years old. *c.* centrum hollowed out by marrow spaces; *i.v.c.* intervertebral cartilage; *m.* marrow spaces; *nch.* notochord; *sy.v.* synovial cavity between two successive vertebrae.

At and after metamorphosis the vertebral column (independently of the atrophy of the tail) becomes shortened owing to the intervertebral discs bending inwards so as to compress the notochord and thus drawing the vertebral regions more closely together. No intravertebral cartilage is found such as occurs in Urodela. Ossification really begins after metamorphosis. As shown in Fig. 31 in the young toad the basidorsals are still entirely cartilaginous. By the time the young frog is 2 years old perichordal ossified centra are formed, but they still enclose a large amount of notochord and the bone is only a thin crust on their external surfaces; their interior is occupied by large narrow spaces. The cartilaginous intervertebral discs are beginning to be cut into by invading connective tissue (Fig. 32). By the time the frog is 3 years old ossification has proceeded so far that only a slender strand of notochord is left inside the centrum; the intervertebral cartilage is almost completely divided into cup and ball, the two being connected only in the axial line by a strand of tissue and ossification of both cup and ball has begun (Fig. 33). The frog becomes mature at 4 years of age. The New Zealand toad, *Liopelma*, retains undivided intervertebral discs throughout life.

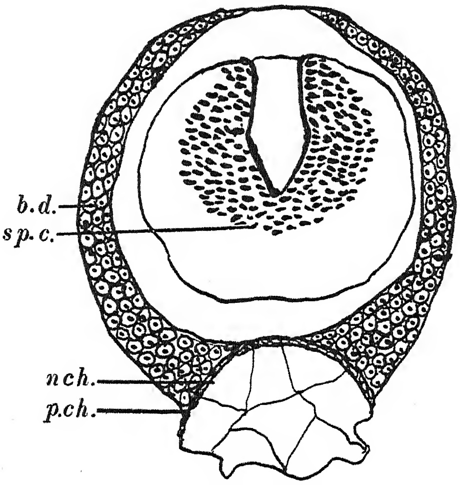

Fig. 34.

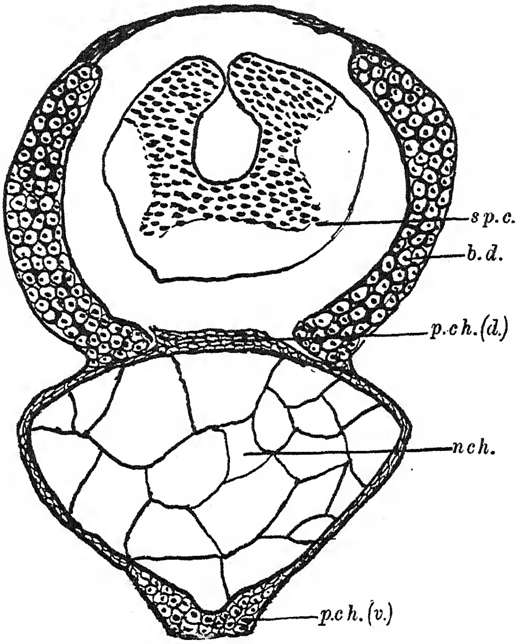

Fig. 35.

Fig. 34. Transverse section through a tadpole of *Bombinator igneus* 12 mm. long. *b.d.* basidorsal cartilage; *nch.* notochord; *p.ch.* perichordal layer of cartilage confined to the dorsal aspect of the notochord; *sp.c.* spinal cord.

Fig. 35. Transverse section through the trunk of a late tadpole of *Xenopus laevis* just before complete metamorphosis. *b.d.* basidorsal cartilage; *nch.* notochord; *p.ch.* (*d*), dorsal thickening of the perichordal layer of cartilage; *p.ch.* (*v*), ventral thickening of the same layer; *sp.c.* spinal cord.

Bufo differs from *Rana* in the rapidity of the metamorphosis and in some minor points in the vertebral column. Thus the 9th vertebra in *Rana* is opisthocoelous, whereas in *Bufo* it is procoelous.

The great difference in the development of *Bombinator igneus* from that of

Rana and *Bufo* is that, when the perichordal tube is formed, only its dorsal portion is converted into cartilage (Fig. 34), the rest of it remains thin and membranous. When metamorphosis occurs the bony centra are formed only from the dorsal portion of the perichordal sheath; its ventral portion and the whole of the notochord disappears. This is what is termed the "epichordal" method of forming the vertebra, in contradistinction to the "perichordal" method which obtains in *Rana* and *Bufo*. The first vertebra is, however, formed perichordally, the whole of the sheath entering into its formation. The hypochord remains fibrous till it is converted into bone. At the tips of the 2nd, 3rd and 4th pairs of diapophyses there are distinct cartilaginous pads which are the rudiments of ribs. Behind the enormously expanded diapophyses of the 9th vertebra, which articulate with the ilium, the 10th vertebra bears a small pair of diapophyses.

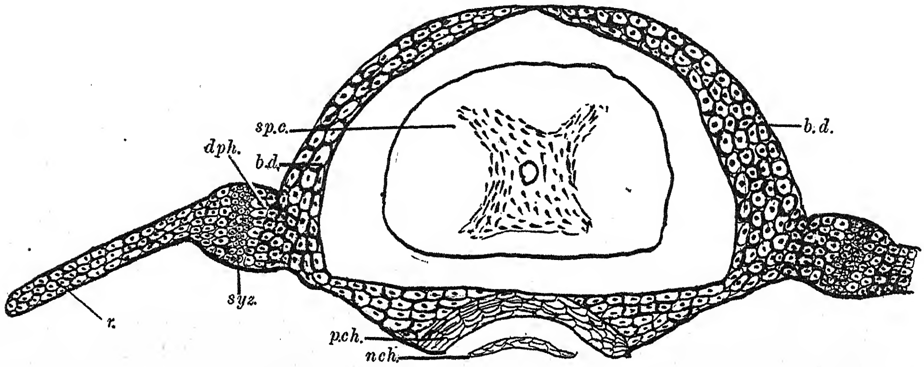

Fig. 36. Transverse section through the trunk of a young *Xenopus laevis* just after metamorphosis. *b.d.* basidorsal cartilage; *dph.* diapophysis; *nch.* shrunken remains of the notochord; *p.ch.* perichordal layer; *r.* rib; *syz.* rudimentary joint between the rib and the diapophysis.

The development of *Xenopus* bears in many respects a strong resemblance to that of *Bombinator*. But in the tadpole (Fig. 35) the perichordal sheath is converted into cartilage all round, and along both dorsal and ventral aspects becomes a thickened rod of cartilage. The ventral thickening was mistaken by Ridewood for the hypochord, a mistake all the more excusable since the true hypochord as in *Bombinator* remains fibrous and inconspicuous until it ossifies. At metamorphosis, however, the whole lateral and ventral regions of the perichordal sheath and its enclosed notochord disappear, leaving only the dorsal portion, which as in *Bombinator* gives rise to the centra. As in *Bombinator* the first vertebra is formed from the whole of the perichordal sheath and the 2nd, 3rd and 4th pairs of diapophyses carry attached to their apices rods of cartilage which are the rudiments of the ribs. Between these ribs and the diapophyses are incipient sutures consisting of softened small-celled cartilage (Fig. 36). These sutures disappear when both diapophysis and rib are co-ossified into one piece of bone.

Reviewing the development of Amphibia as worked out by Mookerjee, we see that the vertebrae are neither arcocentrous nor chordacentrous but *perichordal*. The sharp distinction between the perichordal sheath and arch tissue is clearly seen in

Figs. 29 and 31; although both consist of cartilage, the cartilage has a totally different aspect in the two cases. The only part that the arches take in building the centrum occurs in *Rana* where, between the bases of the basidorsals, a narrow bridge of tissue develops in the front and posterior portions of the centrum, but not in the middle.

The urostyle has nothing to do with the tail. It is a special strengthening of the vertebral column, especially of the basiventrals, designed to resist the pull of the powerful muscles connected with jumping. The ribs are vestigial because of the enormous development of the ventral abdominal muscles as compared with the dorsal muscles. The only arcualia besides the basidorsals which are developed in Urodela are the supradorsals, and, in the tail, the basiventrals. What Gadow mistook for dorsal and ventral intercalaries are the intervertebral discs. In Anura the supradorsals disappear and the basiventrals are only represented by the hypochord. Gadow's suppositious "interdorsals" are again only the intervertebral discs.

IV. AMNIOTA.

When we come to discuss the development of the vertebral column of birds, we pass from Amphibia to Amniota; it will be remembered that Gadow asserted that the vertebrae of Amniota were formed in a totally different manner from those of Amphibia. It remains to be seen how far this assertion is borne out by the researches of Piiper (1928) on the development of the vertebral columns of the bird and the ostrich and those of Dawes (1930) on the vertebral column of the mouse.

1. AVES.

In Amniota the development as is well known is entirely embryonic and Piiper's material consisted of a very complete series of the embryos of the gull (*Larus canus*) from the 2nd to the 10th day of incubation, which he collected himself, and of embryos of the ostrich (*Struthio australis*) belonging to five stages of development, collected in South Africa by Prof. Duerden and sent to Prof. Piiper.

In a gull embryo 2 days old the hinder end of the notochord passes into an undifferentiated mass of tissue behind which lies the open blastopore (neurenteric canal). It is from this mass that the tail later grows out and it is termed the *tail blastema*. The myotomes are compact masses of cells and each is surrounded by a thin cuticular membrane. The ventral sclerotomes are likewise compact hollow masses of cells, each surrounded by a thin cuticular membrane, the *sclerotheca*. This is a surprisingly primitive condition of affairs to find in a Vertebrate so high in the scale as a bird. To find hollow sclerotomes recorded elsewhere we have to go back to Amphioxus. But it is possible that they occur in the herring. Ramanujam's series of embryos was incomplete but in one of them he found a cavity inside a mass of sclerotome cells, and this stage may occur regularly. In Amphibian development there is a curious unexplained tendency for structures represented by epithelium elsewhere to be developed as loose mesenchymatous tissue, which may account for the absence of a sclerocoele in them.

In the gull although both sclerotomes and myotomes are hollow their cavities do not communicate with each other. The sclerotomes extend upwards between the spinal cord and notochord on the one hand and the myotome on the other. They are

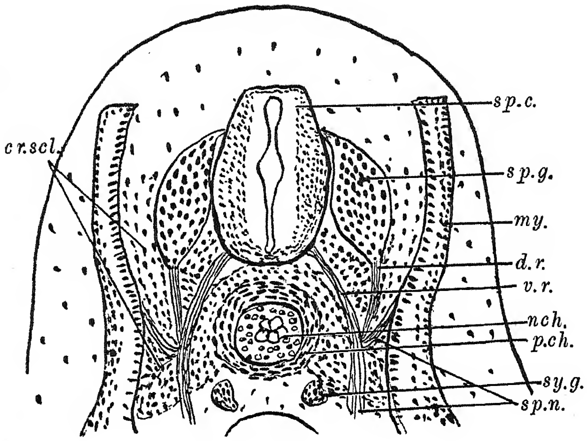

Fig. 37.

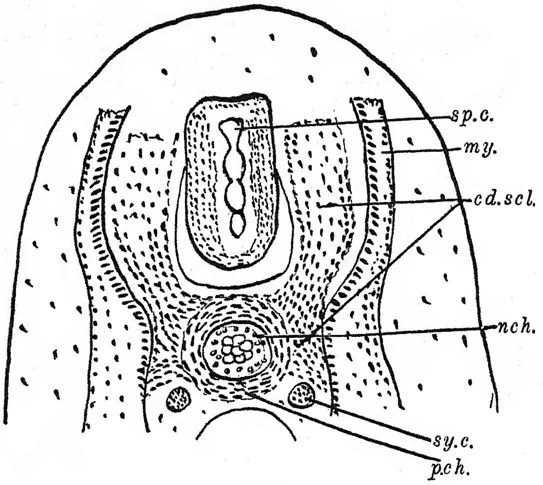

Fig. 38.

Figs. 37, 38. Two transverse sections through the cervical region of a 3-day gull embryo. Fig. 37 is the more anterior. *cd.scl.* caudal sclerotomite; *cr.scl.* cranial sclerotomite; *d.r.* dorsal root of the spinal nerve; *my.* myotome; *nch.* notochord; *p.ch.* perichordal ring of mesenchyme cells; *sp.c.* spinal cord; *sp.g.* spinal ganglion; *sp.n.* spinal nerve; *sy.c.* sympathetic cord; *sy.g.* sympathetic ganglion; *v.r.* ventral root of the spinal nerve.

separated from their predecessors and successors by narrow clefts, the *intersclerotomic fissures*. Their cavities become obliterated dorsally, but are retained ventrally and are termed by Piiper the *intrasclerotomic fissures*. By these latter fissures the sclerotome is divided into a *cranial sclerotomite* in front and a *caudal sclerotomite* behind and impinging on the cranial sclerotomite is the large spinal ganglion (Fig. 37). The upper part of the caudal sclerotomite is the rudiment of the *basidorsal* (Fig. 38). The intersclerotomic fissure soon disappears, the sclerotomes becoming united across it by protoplasmic bridges leaving only space for an intersegmental blood vessel. So it comes about that the cranial sclerotomite of one segment becomes joined to the caudal sclerotomite of the segment in front, and in this way a "re-segmentation" is brought about exactly as in Amphibia.

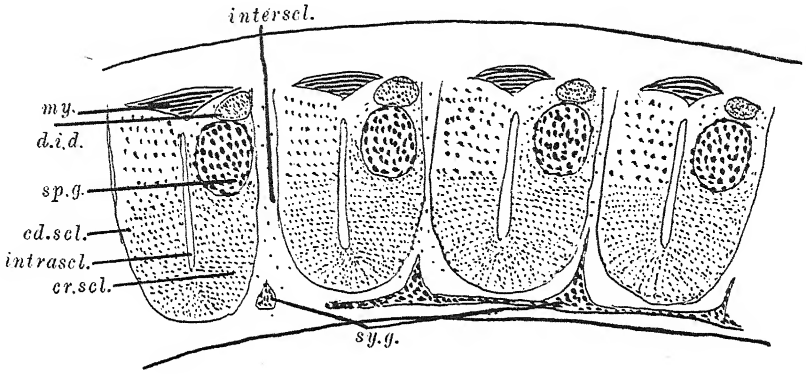

Fig. 39. Parasagittal section through the cervical region of a 4-day gull embryo. *cd.scl.* caudal sclerotomite; *cr.scl.* cranial sclerotomite; *d.i.d.* dorsal interdorsal; *interscl.* intersclerotomic fissure; *intrascl.* intrasclerotomic fissure; *my.* myotome; *sy.g.* sympathetic ganglion.

As in Amphibia the true chordal sheath which consists of elastica externa and interna is not penetrated by cells and remains membranous, but from the inner surfaces of the sclerotomes opposite the notochord there is a profuse budding off of loose mesenchyme which forms an outer investment for the notochord. It is especially thick opposite the middle of the sclerotome, where it constitutes a definite perichordal ring (*p.ch.* Figs. 37 and 38) which obviously corresponds to the perichordal ring and later intervertebral disc of Amphibia.

The corresponding right and left cranial and caudal sclerotomites fuse with one another beneath the notochord. The cranial bridge gives rise to two median bodies separated from one another by a slight groove; these are the rudiments of the *interventrals* (ventral intercalaries). The caudal bridge similarly gives rise to two somewhat larger median bodies;

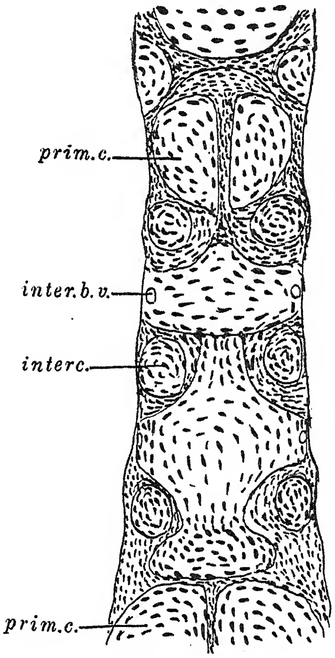

Fig. 40. Frontal section through the lumbar region of the vertebral column of a 6-day gull embryo. *inter.b.v.* intersclerotomic blood vessel; *interc.* intercentrum; *prim.c.* primary centrum or perichordal ring divided ventrally by a longitudinal fissure.

these are the rudiments of the *basiventrals*. The interventrals of one sclerotome and basiventrals of the sclerotome in front adhere together and are termed by Piiper an *intercentrum*. Above the spinal ganglion there is a compact mass of mesenchyme which about the 6th day becomes changed into procartilage. This, termed by Piiper the *dorso-interdorsal*, gives rise to the postzygapophysis of the neural arch, and corresponds to the wing-like outgrowths of the supradorsal in Urodela (Fig. 39). According to Piiper the prezygapophysis is formed later as an independent outgrowth of the anterior aspect of the neural arch.

The intercentra formed from the subnotochordal bridges are single and median in the cervical region; in the thoracic region they are divided into right and left portions by a median groove and in the lumbar region they become completely double (Fig. 40). The centra are formed about the 6th day of incubation by the conversion of that part of the fibrous sheath of the notochord which intervenes between two perichordal rings into cartilage (Fig. 41). These perichordal rings constrict the notochord to a certain extent, although not nearly so much as do the intervertebral discs of the Amphibia; but when the vertebral centra are formed (Fig. 41, *prim.c.*) these centra constrict the notochord a great deal more than do the intervertebral discs. The perichordal ring then forms what Piiper called an *interstitial* body; by the 10th day it has become differentiated into three parts. The anterior and posterior portions become cartilage and the central portion becomes the suspensory ligament. The anterior portion becomes added to the centrum in front as its *opisthospondylous zone* and the posterior portion joins the centrum behind as its *prospondylous* zone. This is exactly what happens in Amphibia, but the change which takes two years to complete in the frog is accomplished in the bird in 10 days. In this way the "secondary vertebra" is formed. The *prospondylous* zone of the vertebra behind and the *opisthospondylous* zone of the vertebra in front enclose between them the joint cavity; how this joint cavity is formed Piiper did not observe; he was not then acquainted with Mookerjee's

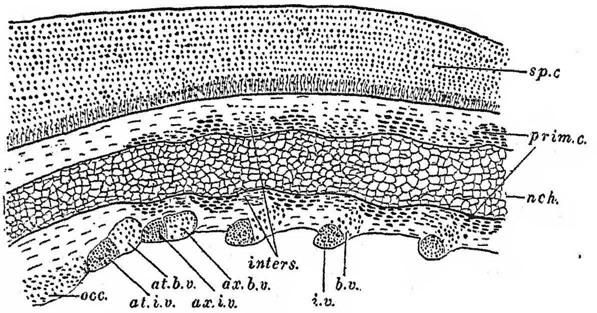

Fig. 41. Median sagittal section through the cervical region of an 8-day ostrich embryo. *at.b.v.* atlas basiventral; *at.i.v.* atlas interventral; *ax.b.v.* axis basiventral; *ax.i.v.* axis interventral; *inters.* interstitial body; *nch.* notochord; *occ.* basioccipital cartilage; *prim.c.* primary centrum (perichordal ring or primary vertebral body); *sp.c.* spinal cord.

work. In the cranial sclerotomite below the spinal ganglion there is a mass of mesenchyme which about the 9th day turns into cartilage and joins the neural arch (basidorsal) in front and the centrum beneath. This cartilage is termed by Piiper the *interdorsal* and obviously corresponds to the dorsal intercalary of the Elasmobranch which Gadow asserted to be absent in all Amniota. The posterior part of the interventral body then becomes converted into ligament. This is the intervertebral ligament; it lies external to the suspensory ligament. Returning to the consideration of the intercentra, they all become converted into procartilage and obviously represent the so-called "subvertebral wedge-bones" which underlie the vertebral column in *Sphenodon* and persist throughout life. In the gull and ostrich the first intercentrum joins the first pair of basidorsals and forms the atlas ring. The anterior interventral part of this intercentrum joins the occipital cartilage. The first primary centrum forms the odontoid process and the second intercentrum tends to bind this centrum to the second or axis centrum.

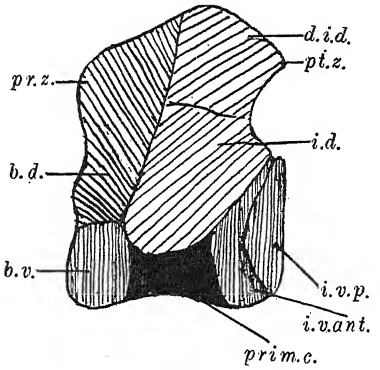

Fig. 42.

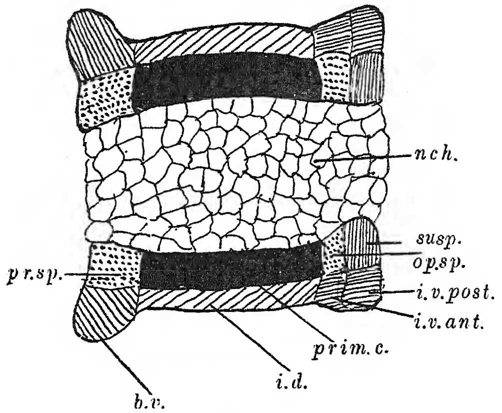

Fig. 43.

Fig. 42. Diagrammatic side-view of the embryonic vertebra of a bird to show the elements of which it is composed. *b.d.* basidorsal; *b.v.* basiventral; *d.i.d.* dorsal interdorsal; *i.d.* interdorsal; *i.v.ant.* and *i.v.p.* anterior and posterior portions of the interventral; the posterior portion forms the intervertebral ligament; *prim.c.* primary centrum, the primary vertebral perichordal ring; *pr.z.* prezygapophysis; *pt.z.* postzygapophysis.

Fig. 43. Diagrammatic frontal section of the vertebra of an embryonic bird in order to show the elements of which it is composed. *b.v.* basiventral; *i.d.* interdorsal; *i.v. ant.* and *i.v. post.* anterior and posterior portions of the interventral: the posterior portion becomes intervertebral ligament; *nch.* notochord; *op.sp.* opisthospondylous zone; *prim.c.* primary centrum (perichordal ring); *pr.sp.* prospondylous zone; *susp.* suspensory ligament.

In the more posterior intercentra, as we have seen, the interventral element degenerates into the intervertebral ligament but the basiventral element joins the vertebra behind it with the vertebra in front of it and grows out into the head of the rib. The rib is connected with the neural arch by a band of connective tissue which becomes later the *tubercular process* or diapophysis of the vertebra.

In Piiper's latest stage, although no ossification whatever had appeared, all the essential features of the Bird's vertebral column had been mapped out, including the fusion of the caudal vertebrae to form the *pygostyle*.

2. Mammalia.

Dawes's paper (1930) on the development of the vertebral column in the mouse is important on account of the closely connected series of stages of the development which he was able to obtain. Owing to the small number of eggs produced at one time and to the development within the maternal womb, it is notoriously difficult to obtain a set of embryos closely linked together in age in the case of any Mammal. Dawes, however, by selecting the mouse, which is an exceptionally favourable subject, and by extensive breeding experiments, was able to overcome this difficulty and his embryos, which belonged to thirty distinct stages of development, ranged from a size of 2 mm. in length up to the new-born young.

The development of the vertebral column of the mouse resembles in its fundamental characters that of the gull, and this resemblance bears strong testimony to the essential unity of the group Amniota. In Dawes's youngest embryo the notochord was a slender cord of cells and extended backwards, ending in a flat plate of cells immediately in front of the blastopore (neurenteric canal) exactly as it does in the gull. The notochord, however, is proportionately much more slender and in a more vestigial condition than it is in the bird. The myotomes which are already formed have in this stage budded off large diffuse sclerotomes which lie between them and the spinal cord and the sclerotomes are separated from one another by *intersclerotomic fissures*. The small rudiments of the spinal ganglia lie about half-way up the sides of the cord. In front of the region where the two roots of the dorsal aorta unite with one another there are on each side no less than five myotomes smaller than the rest. Each gives rise to a sclerotome consisting of very loose tissue but only the last has a small spinal ganglion associated with it and only the last four receive

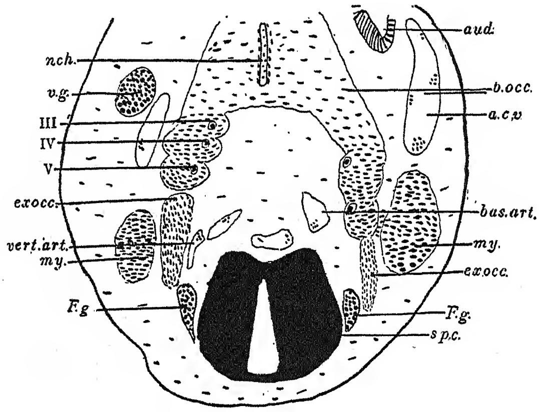

Fig. 44. Section through the occipital region of the skull of an embryo of the mouse 6·5 mm. in length. The section in its front portion is frontal but owing to the curvature of the neck its hinder portion is transverse. *a.c.v.* anterior cardinal vein; *aud.* auditory vesicle; *bas. art.* basilar artery; *b.occ.* basioccipital cartilage; *F.g.* Froriep's ganglion; *ex.occ.* exoccipital cartilage; *my.* myotome; *nch.* notochord; *sp.c.* spinal cord; *vert.art.* vertebral artery; *v.g.* vagus ganglion; III, IV, V, nerves issuing from the three posterior hypoglossal segments.

spinal nerves from the cord. In the embryo of the calf Wilson (1925) has described small ganglia on the last three of these nerves. In Fig. 44 on one side of the section three of these sclerotomes are seen. In front of the first of these myotomes there is an unsegmented region of mesoderm which reaches to the auditory vesicle. In this region is found the vagus ganglion and it is this part of the head which corresponds to the occipital region of Amphibia.

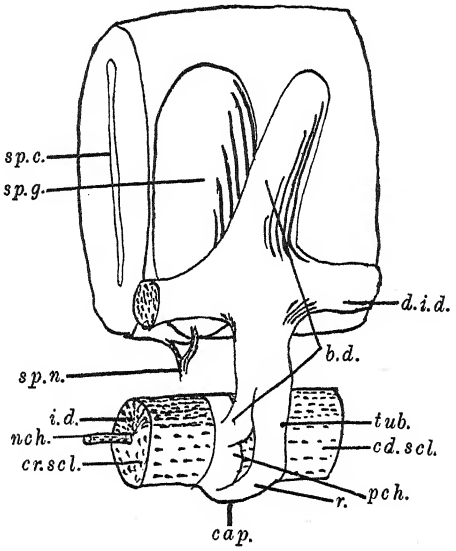

Fig. 45.

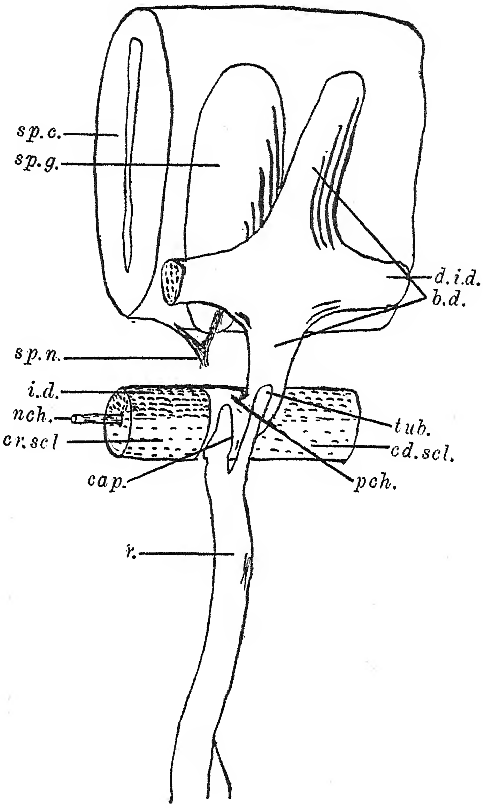

Fig. 46.

Figs. 45, 46. Reconstruction of a cervical and thoracic segment respectively of a 6·5 mm. embryo of the mouse viewed from the side. *b.d.* basidorsal; *cd.scl.* caudal sclerotomite; *cap.* capitulum of the rib; *cr.scl.* cranial sclerotomite; *d.i.d.* dorsal interdorsal; *i.d.* interdorsal; *nch.* notochord; *pch.* primitive intervertebral body or perichordal ring; *r.* rib; *sp.c.* spinal cord; *sp.g.* spinal ganglion; *sp.n.* spinal nerve; *tub.* tubercle of rib.

When the embryo reaches the length of 4 mm. a definite "intrasclerotomic fissure," *i.e.* a cavity, appears in each sclerotome and it thus becomes divided as in the bird into cranial and caudal sclerotomites, but this cavity does not appear in the five rudimentary anterior myotomes. The spinal ganglion divides the cranial sclerotomite into an upper and lower half; the caudal sclerotomite is undivided but the cells in its upper part are regularly arranged, whereas those in the lower part are irregular. The upper part is the rudiment of the *basidorsal*, whilst the lower part is the beginning of the *basiventral*. In the cranial sclerotomite the tissue above the spinal ganglion becomes condensed and eventually gives rise to the *dorsal-interdorsal* out of which the post-zygapophysis develops: the tissue below the ganglion above the notochord is the rudiment of the *interdorsal*. Below the notochord there is a mass of loose tissue which eventually forms the interventral. According to Gadow it is the interventral which in the Mammal and in Amniota generally gives rise to the centrum, the interdorsal being absent. Dawes's results are in complete contradiction to these assumptions. The notochord becomes invested, as in the bird, by a series of thick perichordal rings of cells budded chiefly from the inner margins of the basiventral region of the caudal sclerotomite but partly from the hinder part of the cranial sclerotomite. The rings correspond of course to the "interstitial bodies" of the bird and to the "intervertebral discs" of the Amphibia. But in the tissue spanning the *intersegmental* fissure which has now almost disappeared, a violet-staining intercellular matrix appears. This tissue assumes the form of a **U** underlying the notochord; it is derived from cells budded from the hinder part of the basiventral and the front part of the interventral belonging to the segment behind and thus these rudiments alternate with the perichordal rings. These masses are the first rudiments of the *centra*; the peculiarity of the Mammal is that they do not, as in the other groups, arise as cylinders, but as **U**'s with a dorsal gap. The bending of the neck (cervical flexure) reaches its maximum when the embryo has attained a length of 7 mm. This bending crushes the rudimentary segments in front together into a wedge-shaped mass: the junction of head and neck is marked by a fissure in the sclerotomic tissue which is the intersclerotomic fissure between the last of the rudimentary segments and the first fully formed cervical segment. The interdorsal of this segment together with the loose tissue below it forms a cartilage, the "*pro-atlas*," which intervenes between the 1st cervical vertebra and the basi-occipital cartilage. The cervical basidorsal becomes detached from the perichordal disc below it and attached to the incipient cervical centrum. The basiventrals grow out into the fissures between the myotomes and give rise to the rudimentary capitula of the ribs. Loose strands of tissue represent the tubercula, which of course are connected with the basidorsals. In the cervical region the capitula become attached to hinder aspects of the perichordal discs in the thoracic region to their sides. The atlas segment differs from the others in that the basidorsals do not become detached from the perichordal ring, and that the ventral part of the atlas ring is formed from the capitula of the vestigial atlas ribs; that is, from the first pair of basiventrals.

When the embryo has reached 8 mm. in length the 5 rudimentary somites of the head have become consolidated into a mass of tissue which is being chondrified and forms the basi-occipital cartilage. It is far in advance in its development of the pro-atlas or atlas. The second of these somites has lost its nerve, but the last three somites retain their nerves; these form the roots of the hypoglossal. The ganglion developed in relation to the last somite becomes Froriep's ganglion (Fig. 44) and is eventually attached to the 11th cranial nerve. In the cervical region the basidorsals become detached from the perichordal discs below them and fused on their hinder aspects both with the centrum and with the interdorsals which are now chondrified. Thus a certain portion of what may be called the secondary centrum is derived from the tissue of the basidorsals; the interdorsals fill up the dorsal gap in the primitive U-shaped centrum. Thus the complete centrum acquires components from the basidorsals and basiventrals of the segment in front and from the interdorsals and interventrals of the segment behind it. Inside each perichordal disc the notochord dilates and forms a swelling which, as the "nucleus pulposus," persists for life. There is a "nucleus pulposus" in the perichordal disc which separates the atlas and pro-atlas and the latter has now developed a well-marked U-shaped primitive centrum. This centrum eventually becomes fused with the tip of the odontoid process. In the thoracic region, of course, the ribs are long and extend downwards into the myocommata. The perichordal disc becomes differentiated as in birds into an opisthospondylous zone in front, a prospondylous zone behind and a ligament between. The prospondylous and opisthospondylous zones become the *epiphyses* of the centra and the ligament between contains the nucleus pulposus. The basiventrals of the thoracic region become connected with each other beneath the primary centrum and this bar of tissue becomes added to the secondary centrum. There is, even in the new-born mouse, a *nervus-sub-occipitalis* devoid of a spinal ganglion passing through an aperture in the arch of the atlas in front of the first persistent cervical spinal nerve.

V. CONCLUSIONS.

If we now take a general survey of all the types of development of the vertebral column which we have studied we find that they agree in their fundamental features. Leaving out the Cyclostomata, the cartilaginous Ganoids and the Dipnoi in which an undivided cartilaginous tube invests the notochord throughout life, we find that the centra are formed as perichordal rings of cells which alternate with the myotomes. The primitive arcualia—basidorsals, basiventrals and ribs—are always developed in the myocommata. The notochord is always invested by two sheaths, for even in Amphioxus it receives an investment outside the true chordal sheath from the inner wall of the hollow sclerotome. In Elasmobranchs and in the more primitive Teleostei the inner portion of the centrum is derived from the true chordal sheath which becomes invaded by mesenchyme cells, chondrified and calcified (Fig. 47 A). In all the land vertebrates the rings forming the centra alternate with thicker rings of cells opposite the middle of the myotomes. Except in the Mammalia, these intervertebral rings constrict and eventually destroy the notochord and then divide into cup- and ball-like joints (Fig. 47 C, D, E). In the Mammalia they remain thin and enclose a persistent notochord, and this condition can only be compared with that of the Teleostean fish where also the vertebrae are separated by a persistent notochord. This, like the skin glands, is one of the extremely primitive features retained by the Mammalia which shows that they must have very early diverged from the other Amniota.

Wherever in Vertebrata there is persistent pull of the muscular tissue, the connective tissue to which it is attached and which is pulled on tends to become first chondrified and eventually ossified; this accounts for the situation of the arcualia in the myocommata.

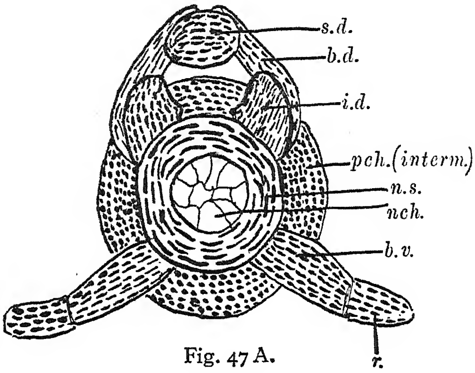

Fig. 47 A.

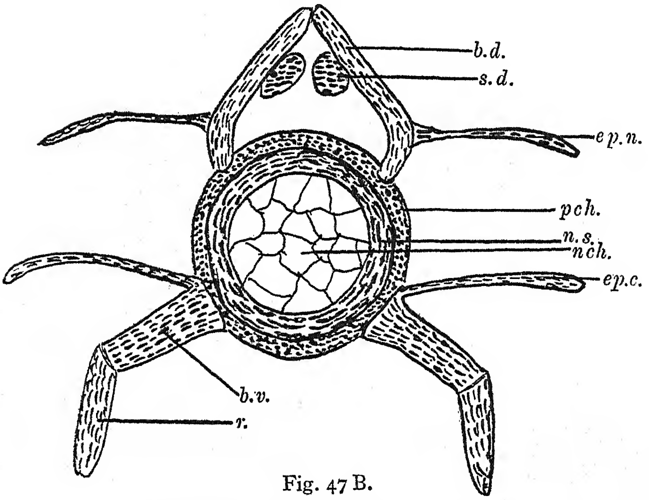

Fig. 47 B.

Fig. 47. Diagrammatic thick sections through the vertebrae of six typical Vertebrates seen from behind. *b.d.* basidorsal (= neural arch); *b.v.* basiventral represented either in cartilage or connective tissue; *cap.* capitulum (head of rib); *dph.* diapophysis (transverse process); *ep.c.* epicentral bone; *ep.n.* epineural bone; *i.d.* interdorsal (= intercalary); *nch.* notochord; *n.s.* notochordal sheath; *pch.* (*interm.*) perichordal sheath (= intermedialia); *r.* rib; *pt.z.* postzygapophyses; *s.d.* supradorsal (zygapophyses); *syn.* joint; *tub.* tubercle of the rib.

Fig. 47 A is a section through the vertebra of an Elasmobranch. The supradorsal is represented as median and single but in other genera it may be paired. The interdorsals stand freely out from the vertebra and are known as intercalaries. The inner or true chordal sheath is very thick and forms at least half the centrum and on it the arches rest. The outer or perichordal sheath is represented by four great wedges alternating with the arch bases termed intermedialia. The rib which extends horizontally outwards is the segmented end of the basiventral.

Fig. 47 B is a section through the vertebra of the herring. Both chordal and perichordal sheaths take part in building up the centrum and both later become ossified. The supradorsals are paired and adhere to the inner aspects of the basidorsal. The ribs, the segmented off ends of the basiventrals, extend downwards, not outwards, but from the base of the basiventral another bone, the epicentral, extends outwards in the position of the Elasmobranch rib. An epineural bone extends outwards horizontally from the basidorsal.

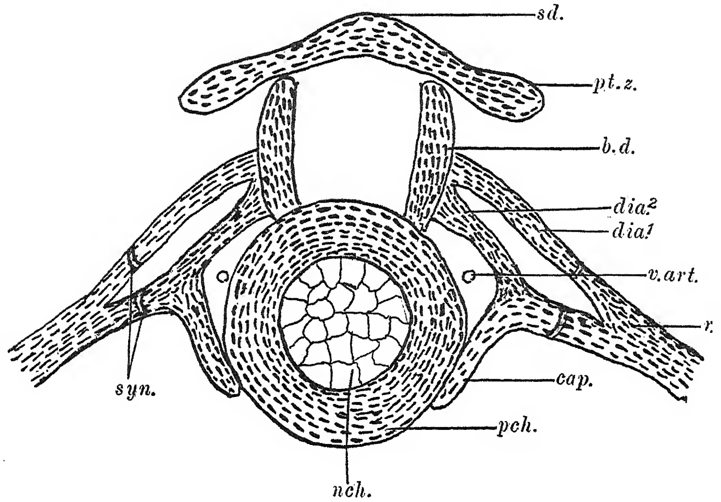

Fig. 47 C.

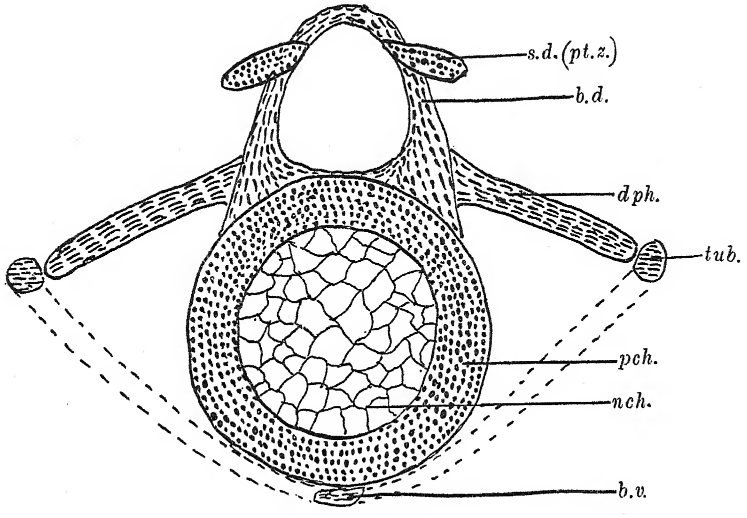

Fig. 47 D.

Fig. 47 C is a section through the vertebra of a Urodele (Triton). The postzygapophysis is seen to be a lateral outgrowth of the supradorsal. The centrum is entirely formed from the perichordal sheath. The rib is attached to the centrum by a capitulum and to the neural arch (basidorsal) by a forked diapophysis. Besides the ribs there are no basiventrals.

Fig. 47 D is a section through the vertebra of an Anuran. The basiventrals are represented by a mass of connective tissue (*b.v.*). The supradorsals are represented by the paired postzygapophyses. The basidorsals meet in the middle line below and are quite distinct from the cartilaginous centrum formed by the perichordal sheath. The ribs are represented by the tubercular processes only adherent to the ends of the unforked diapophyses.

The posterior zygapophyses (dorsal-interdorsals) appear to represent the supradorsals of Elasmobranch fish; these may occur as a median or as paired series of cartilages. What relation if any the zygapophyses of Teleostei bear to their supradorsal cartilages requires to be cleared up, but it is probable that there too the supradorsals give rise to the post-zygapophyses. In Urodela, as we have seen, there is a median series of supradorsals which give off the zygapophyses behind. The term "parapophysis" has given rise to a good deal of misunderstanding. It originally meant (as contrasted with "diapophysis") an outgrowth from the *centrum* to which a rib became attached. But Mookerjee's work has shown that the existence of a flexible joint does not imply that the two pieces joined by the flexible tissue were originally

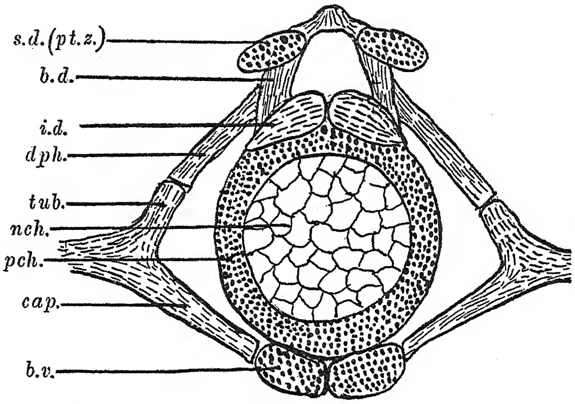

Fig. 47 E.

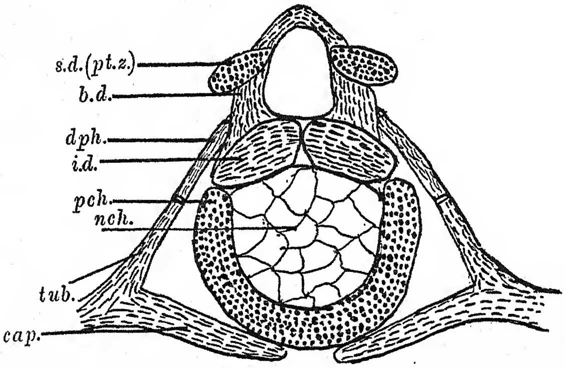

Fig. 47 F.

Fig. 47 E is a section through the vertebra of a gull. The supradorsals (dorsal interdorsals) are paired and form the postzygapophyses. The interdorsals (or intercalaries) become later completely amalgamated with the centrum formed from the perichordal sheath. The basiventrals form with interventrals paired "intercentra" to which the capitula of the ribs become attached.

Fig. 47 F is a section through the vertebra of an embryo mouse. The centrum formed from the perichordal sheath does not completely surround the notochord, the gap between its two arms being filled by the interdorsals. Separate intercentra are not formed.

separate. A joint is often formed at a position of flexure by the cutting in two of an originally single piece by invading connective tissue.

The great difference between Amniota and Amphibia consists in the number of vertebrae included in the skull. The relation of the hinder part of the skull to the vertebral column has been described in the case of the Mammal only because Dawes studied it and Piiper did not; but there is strong reason for believing it to be essentially the same in all Amniota. Another difference between Amniota and Amphibia is that only the former possess a neck, that is a region of the body in which the vertebrae have vestigial ribs and which is devoid of peritoneal cavity.

Dawes's study of the mouse suggests that these two features are due to the same cause, viz. the *cervical flexure* of the embryo which exists only in Amniota. It is this flexure which welds five segments behind the vagus region into a wedge-shaped mass and presses them forwards so that they fuse with the skull in front—and the same flexure pushes back the heart and lungs and renders the ribs in the cervical region functionless (Fig. 48).

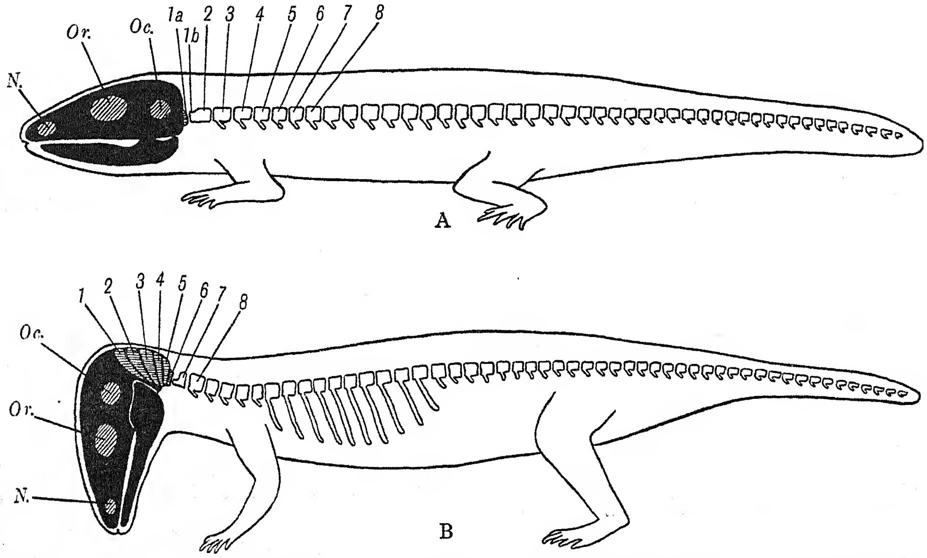

Fig. 48. Two diagrams to illustrate the development of the Amniote skull out of the Amphibian skull. *N.* the nasal sac; *Or.* the orbit; *Oc.* the auditory sac. A. Diagram of a primitive Amphibian showing the skeleton. 1*a*, 1*b*, the two halves of the intercalary vertebra. 1*a* is the front half which becomes the occipital condyles. 1*b* is the hinder half which becomes the cups into which the condyles fit; 2–8 the subsequent vertebrae. B. Diagram of a primitive Amniote showing the skeleton. 1–5 the first five vertebrae which are incorporated with the skull forming the elongated occipital region. 5, the atlas vertebra. 6, the axis vertebra carrying the odontoid process. Note the downward posture of the head and the consequent crushing together of segments 1–5.

What was the origin of this flexure? It seems to us that it must have originally been due to an alteration in the habitual posture of the head and in the mode of eating. If we think of the primitive four-footed land vertebrates, which at first were a huge mob of newt-like creatures amongst which it would be difficult to distinguish the ancestors of the modern newts, a certain number seem to have persisted in the primitive habit of darting directly at their prey and seizing it with their jaws. These became the Amphibia. The rest, however, who migrated further inland began to devour the plants beneath them: this necessitated bending the front part of the body downwards and thus both the neck and the lengthened skull were brought into being. It is a remarkable fact that when we employ all the evidence at our disposal to trace back two phyla to their common ancestral stock we find that the original cause of their separation was a change in habits. This has been shown in the case of the stalked and unstalked Echinodermata and in the case of the Mollusca and Annelida, and the same thing now proves to be true of Amphibia and Amniota. Habit changes first and structure a long time afterwards. Habit is the real driving force in evolution.

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CONATION AND PERCEPTION IN ANIMAL LEARNING

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I. INTRODUCTION.

It is clearly impossible within the confines of an article in these pages to summarise the extensive and varied work which has been carried out in recent years on animal learning, and I shall make no attempt to do so. The reader will find adequate summaries and discussions in Washburn (1926) and Tolman (1927, 1928); full references to current work will be found in *Psychological Abstracts*, published by the American Psychological Association at Lancaster, Pa.

The same embarrassment of riches confronts anyone who attempts to give an account of the theories of animal learning. According to Lashley (1929) "the whole theory of learning and intelligence is in confusion," and it is certainly true that the greatest diversity of opinion and point of view exists among the psychologists and the physiologists who have studied the question. A comprehensive review of theories would again take up too much space here.

In these circumstances, I have limited my field to a discussion of one particular line of advance in the theory of animal learning, namely that trend of opinion which lays stress upon the strictly psychological concepts of conation and perception, and emphasises their importance as determiners of learning. The reader should bear in mind that many other types of theory exist—for example, the purely physiological theory of conditioned reflexes—which purport to account for animal learning, and that no one type of theory has received anything like general acceptance. My sole aim has been to interest the biological reader in some recent work carried out from the psychological point of view which seems to me to have significance for general biology. It is unfortunate that a cleavage exists between biology and psychology so great that one side does not know what the other side is doing. There is, for example, much work on animal behaviour of first-class importance to the biologist which is buried in psychological journals that he never sees. I hope that this article will induce and assist the interested reader to study the psychological literature on animal behaviour. An admirable introduction to the subject is Bierens de Haan's little book (1929). The psychological reader will, I hope, pardon the temerity of a mere biologist in invading his domain, and make allowance for his deficiencies.

II. THORNDIKE'S THEORY AND ITS CRITICS.

The experiments which Thorndike (1898, 1911) carried out on the learning process in cats, dogs and chicks in the late 'nineties are justly regarded as classical, and mark a turning point in the history of comparative psychology, for they were practically the first carefully planned and fully controlled experimental studies of the behaviour of the higher animals.

Although it may safely be assumed that they are familiar to all students of the science, it is desirable to recall their main outlines and to restate their results, for the subsequent development of the theory of animal learning has consisted largely in an extension, a criticism and a correction of Thorndike's views.

Thorndike was the inventor of the "puzzle box" method of studying "intelligent" behaviour. He constructed various types of boxes, made largely of wooden slats, which could be opened from the inside, *e.g.* by pulling on a ring attached over a pulley to a bolt, or by turning a button latch, and so on. The details are not important, but it may be noted that the release mechanism was not as a rule completely visible to the animal enclosed in the box, and the how and why of it were probably completely incomprehensible to it. Illustrations and descriptions of these boxes will be found in Warden (1928) and Adams (1929). Using as his experimental subjects chiefly kittens in a state of "utter hunger"—which meant that they had been without food for 14 hours—he determined how long they took to get out of the box in successive trials, giving his results in the form of time curves. A small amount of food was given as a reward after each trial. With two exceptions the thirteen cats tested showed the same typical behaviour, which is vividly described by Thorndike as follows:

> When put into the box the cat would show evident signs of discomfort and of an impulse to escape from confinement. It tries to squeeze through any opening; it claws and bites at the bars or wire; it thrusts its paws out through any opening and claws at everything it reaches; it continues its efforts when it strikes anything loose and shaky; it may claw at things within the box. It does not pay very much attention to the food outside, but seems simply to strive instinctively to escape from confinement. The vigor with which it struggles is extraordinary. For eight or ten minutes it will claw and bite and squeeze incessantly (1911, p. 35).

The time curves showed that, in general, the cat got out more and more rapidly in successive trials; sometimes the curve showed a rapid drop; in other cases there was a gradual descent, say from a time of 300 sec. to a constant 6–8 sec. A rapid drop might mean that the cat had sized up the situation, seen the solution and acted upon it, thus exhibiting "insight." Thorndike, however, definitely rejected this interpretation, holding that the sudden improvement shown in several experiments merely indicated that the problem was so very simple and obvious that a very few experiences sufficed to establish a perfect association. His explanation of how the association came about was along quite different lines. It is summed up in its essentials in the following passage:

> The cat that is clawing all over the box in her impulsive struggle will probably claw the string or loop or button so as to open the door. And gradually all the other non-successful impulses will be stamped out and the particular impulse leading to the successful act will be stamped in by the resulting pleasure, until, after many trials, the cat will, when put in the box, immediately claw the button or loop in a definite way (1911, p. 36).

The animal is, as it were, passively affected by fortuitous successes or failures, which impress *this* act upon it and wash out that other; learning or the formation of associations is regarded as a more or less mechanical process imposed upon the animal; there is no reasoning, no inference, no judgment by results, necessarily implied. To quote Thorndike again:

> The one impulse, out of many accidental ones, which leads to pleasure, becomes strengthened and stamped in thereby, and becomes more and more firmly associated with the sense impression of that box's interior. Accordingly it is sooner and sooner fulfilled. Futile impulses are gradually stamped out. The gradual slope of the time curve, then, shows the absence of reasoning. They represent the wearing smooth of a path in the brain, not the decisions of a rational consciousness (1911, p. 74).

Thorndike does not deny that cats may have memory images or representations, but he considers the evidence for this unsatisfactory and the process of learning explicable without it. From the fact that his cats apparently did not learn by watching other cats release themselves, and that they did not learn by being "put through" the act leading to release, he concluded that the essential thing in their learning was not an association of ideas (of release, of the proper method of escape) but the association of particular sense impressions with particular impulses leading to release and pleasure. He held that no cat could form an association leading to an act unless there was included in the association an impulse of its own. The full association series formed is accordingly: sense impression (situation), impulse, action, pleasure. Impulse, in Thorndike's use of the word, means simply impulse to action, the giving of an innervation; it does *not* mean either consciousness of action, or motive. The association series is, of course, not established without the aid of the last term, pleasure or satisfaction.

Thorndike considered it possible or even probable that "the entire fact of association in animals is the presence of sense impressions with which are associated, by resultant pleasure, certain impulses, and that, therefore, and therefore only, a certain situation brings forth a certain result" (pp. 108–9). His cats did not "think of" getting out in a particular way; after some trials, and as a result of the association formed by the resultant pleasure, the situation—the sight of the box from inside—made direct connection with the appropriate impulse and response. Just what this means can best be realised by taking the analogy of our own action when learning to play a game like tennis. There is here, at least after the first self-conscious attempts, "desire, sense impression, impulse, act and possible representations," just as in the cat learning to escape from a box. Both activities are learned gradually. The tennis player does not explicitly think "here is a ball coming in such and such a direction, I must hit it just in the same way that I hit a similar ball a few minutes ago"; on the contrary, when he is sufficiently experienced, the sense impression of the ball leads at once to the appropriate innervation or impulse which brings the racquet to the right spot with the right angle and force in anticipation of the ball's arrival. Of course, clear percepts and ideas are also present, but abstracting from these, we get a good picture of what may be presumed to be the course of events in the experience of the cat that has learned how to get out of the box.

Before proceeding to state the formal laws of learning propounded by Thorndike in a later paper in his *Animal Intelligence*, it is worth while calling attention to the fact that Thorndike recognises the importance of another factor in learning, namely attention. "Unless the sense impression is focussed by attention, it will not be associated with the act which comes later. Unless two differing boxes are attended to, there will be no difference in the reactions to them. The really effective part of animal consciousness, then, as of human, is the part which is attended to; attention is the ruler of animal as well as human mind" (pp. 144–5, see also p. 249). This does not imply thinking about the object attended to, but readiness to act in relation to it. This element of attention appears to imply a certain activeness of attitude on the part of the animal, of which little account is taken in Thorndike's general theory.

We come now to Thorndike's two laws, of Effect and Exercise, which in his view account for all learning. It is best to quote his own words.

> The Law of Effect is that: Of several responses made to the same situation, those which are accompanied or closely followed by satisfaction to the animal will, other things being equal, be more firmly connected with the situation, so that, when it recurs, they will be more likely to recur; those which are accompanied or closely followed by discomfort to the animal will, other things being equal, have their connections with that situation weakened, so that, when it recurs, they will be less likely to occur. The greater the satisfaction or discomfort, the greater the strengthening or weakening of the bond.
>
> The Law of Exercise is that: Any response to a situation will, other things being equal, be more strongly connected with the situation in proportion to the number of times it has been connected with that situation and to the average vigor and duration of the connections (1911, p. 244).

These laws hold good in Thorndike's opinion for all animals, including man, that have the capacity for learning (but see p. 158 below). Learning is essentially determined by its outcome or issue in satisfying or annoying conditions; "satisfiers" or "annoyers" are then the factors that decide whether learning takes place or not. This general theory of learning is developed further by Thorndike in his *Educational Psychology* (1913–14), with special reference to man. To discuss it fully would be stepping outside the limits of this article; the reader who is interested may be referred also to Koffka's exposition and criticism (1928, p. 96 ff.). Thorndike's latest views (1931) are considered briefly below (p. 159).

It remains to point out that Thorndike considered the connections established between situation and response to be neural in nature. They are represented by

> connections between neurones and neurones, whereby the disturbance or neural current arising in the former is conducted to the latter across their synapses. The strength or weakness of a connection means the greater or less likelihood that the same current will be conducted from the former to the latter rather than to some other place (1911, pp. 246–7).

We have now sketched in the barest outline the general theory of learning as originally proposed by Thorndike; details will be filled in as we proceed to discuss the various criticisms which have been levelled against his methods and deductions.

The earliest of these criticisms was made by Mills (1898, 1899), who pointed out that the subjects used by Thorndike were probably in a panicky condition; he considered the method of confining cats and dogs in small boxes to be quite unsuitable for a study of their normal behaviour. Thorndike did not accept these criticisms as valid (1899).

In 1901 the late Prof. Hobhouse discussed Thorndike's theory at some length and reported some further experiments of his own which were undertaken largely to test Thorndike's conclusions (1901, 1915 and 1926). Taking first of all the time curves, he pointed out that many of them did not show the gradual descent which Thorndike obviously regarded as typical; several cats and dogs appeared to have learned the trick of getting out after one or two trials, and that in problems by no means easy. He considered that Thorndike's own results proved that dogs and cats do not invariably learn by habituation, but that, on the contrary, at least some of them learn by concrete experience, or appreciation of results. He adduced evidence that some animals, including cats, *can* learn by being put through the appropriate action, contrary to Thorndike's assertion. His most important contribution is, however, the relation of a number of experiments tending to show that an animal can learn from watching an action carried out by the experimenter.

> If an animal pulls a string because having done so before it has given him pleasure, it is possible to regard his education as the gradual growth of a random way of acting into a habit. But if he pulls it because he has seen it pulled, and then got the pleasant result, his act appears rather as a practical application of what he has seen—a perceptual relation converted into a practical adjustment (1926, p. 185)

—and Thorndike's theory breaks down.

We need mention only a few of Hobhouse's results. His dog Jack learned at the fourth trial to pull up a small bucket containing meat which was suspended by a string from the banisters of a staircase landing. The string was attached to one banister and passed round the next, so that the dog had to pull at the string between the two, letting it run through its teeth so as to bring the bucket up near its mouth. Hobhouse showed the dog how to do the trick, attracting its attention to the string and how it worked. He ascribed the dog's success to "a fusion of the method of perception of results with that of experience of success and failure." It has to be added that Jack had previously learned the trick of pulling down a string attached to a card on which meat was placed. In another experiment he learned to open a cage or a box containing meat by pressing on a lever, solving the problem after three to six trials. Hobhouse notes that he did not appear to discover the trick by accidental clawing. He naturally aimed straight at the food and had to learn to claw or bite—for he used both methods—at the lever some inches away. Hobhouse records the significant fact that he seemed to learn the right action definitely and suddenly, though he sometimes forgot the solution afterwards. He learned also to open a box by pulling out a bolt, and so did a cat—much more neatly than the dog. More complicated problems involving a lever as well as a bolt were also solved.

Hobhouse concluded from these and many other experiments with dogs, cats, elephants, otters and monkeys that his animals were guided by seeing him perform the trick, that they learned therefore, or were influenced in their learning, by perception of results.

> There was in nearly all the experiments which succeeded (and most did succeed) a certain point at which a well-marked change of attitude took place. This point varied, from the second trial to the seventh or eighth, and once or twice it was still longer delayed. Before this point, the efforts were of a random character: not purposeless, indeed, but directed towards getting the foods by the methods natural or habitual to the animal. At a certain point it became clear that the animal was abandoning these methods and adopting mine. The transition was more or less clearly marked, in accordance with the nature of the thing to be done. Where that was something very definite, the transition was striking and conspicuous (1926, p. 231).

The movements to be carried out were in some cases complicated, requiring definite sequence, persistence and adjustment; this goes to indicate, in Hobhouse's view, that the animal had a "practical idea" of what it was about, that it was directing its action towards bringing about a definite change in the situation it perceived, with a view to achieving its end or goal. The change from diffuse to directed effort took place after the "critical success," which was usually the first success. Relatively articulate or free ideas—as shown, for example, by their use of diverse objects as tools—were possessed by monkeys, the behaviour of which was also studied by Hobhouse.

Hobhouse's experiments, though somewhat elementary, have considerable importance historically, inasmuch as they enabled him to point out certain features of animal learning which were not allowed for in Thorndike's theory. They were published first in 1901.

They seem to have attracted comparatively little attention among comparative psychologists, but they were utilised in the acute and subtle criticism of the Thorndike theory by Stout, to which we must now turn.

Stout (1913) agreed that Thorndike's results could be explained without ascribing to the animals any free or explicit ideas; the learning took place at the perceptual and not at the ideational level. He considered, however, that Thorndike had given insufficient weight to the important factor of attention; Hobhouse's results showed that *if the animal's attention were secured* it could learn by watching the trick performed, and the growth of a general disposition to attend and imitate was clearly indicated in his experiments. Stout held that there was no reason for doubting

> that causal inference of a rudimentary kind is shown in the appreciation of success and failure, in persistency with varied effort, in the repetition of ways of behaviour which have yielded satisfactory results in the past to the exclusion of others, and also generally in the anticipation of like results when like conditions occur (p. 381).

He pointed out that Thorndike's idea of a direct connection between a certain sense impression and the innervation of a certain group of muscles was not an adequate account of the facts. What actually happens is that the animal learns to produce a certain effect by any means in its power. Hobhouse had already called attention to the fact that the method of solution was by no means invariable—the cat, for instance, might pull down the loop by means of its paws on one occasion and with its teeth on the next. Referring to the analogy with tennis playing which Thorndike adduced as a parallel to animal learning on the perceptual level, he pointed out that the actions of a tennis player could certainly not be reduced to more or less mechanical associations between impressions and actions—there was in addition keen attention on the part of the player and constant adjustment of action to the rapidly changing sense impressions. Thorndike's theory could only hold good in cases where action had become fixed and automatic through much repetition, and attention had ceased to be necessary. Even Thorndike finds great difficulty in imagining how the satisfaction which comes at the end of a successful train of action can work back to reinforce the connection; in Stout's view of the learning process this presents no difficulty.

> The unity and continuity of interest which binds a sequence of distinct acts into a single action has its counterpart on the side of retentiveness in the formation of a cumulative disposition. On the first occurrence of the process the traces left by prior phases persist in and contribute to determine succeeding phases. They unite in a single cumulative disposition. When the activity is repeated, whatever stimulus prompts it re-excites the total cumulative disposition left behind by its previous occurrence. The cumulative disposition has been modified in the anterior experience, and accordingly the re-aroused activity takes a correspondingly modified course as a whole. This is the process which we have described as *revival of acquired meaning*. Without this there can be no learning by past experience; and intelligent learning by experience may be due to it alone (p. 384).

It will be seen that Stout approaches the problem of learning from the strictly psychological point of view, and does not import physiological concepts into his explanation, for by "traces" he does not mean material traces, nor by "disposition" a material configuration.

Koffka (1928) in his very full and interesting treatment of the problems of instinct and learning also deals with the matter primarily as a psychologist, and takes up somewhat the same point of view as Stout, enriched by the new ideas of *Gestalt* psychology. It is characteristic of Thorndike's theory that insight and intention are assumed to play no part in the learning; the elimination of the useless and the perfecting of the useful movements go on without the active participation of the animal; the whole process is mechanical. Koffka challenges this assumption on the basis of Thorndike's own experimental results. Taking first the time curves, he points out that many of them show the sharp fall which would ensue if the animal showed some insight into the problem. Thorndike explains such cases by saying that the problem was very simple, and the solution very obvious. Koffka counters this by pointing out that we do not know whether a problem is simple or not *from the animal's point of view*, and in any case if the whole process is mechanical there is no point in saying that any solution is more obvious than any other. Further, many cases are known, and are indeed common knowledge, in which animals learn after one experience—not only monkeys, but, for instance, chicks and cats.

Koffka further objects to the experiments that they were ill adapted to the capacities of the animal, and such that the animal could neither inspect the mechanism of release nor understand it. The phenomena of "transfer of training," as when a cat trained to pull at a loop at the front of the cage rapidly learns to pull the loop when it hangs at the back, also tell against the mechanical theory of learning; the loop has become a significant element of the perceptual field; it is attended to; it has become for the cat "something to be moved" as a step towards release.. In general, Koffka emphasises the meaningful, purposive character of animal learning, agreeing with McDougall and Tolman that learning is essentially *problem solving*.

We shall consider this view more fully below (section IV). Here we may briefly refer to the criticisms made by McDougall (1923) of the Thorndike theory. He rightly points out that the theory of "stamping-in" is quite inadmissible from the mechanistic standpoint, which is at bottom accepted by Thorndike; it is not conceivable that in a world of pure mechanism the effect should precede the cause. The results are to be interpreted in terms of mind, the function of which is

> to govern present action by anticipation of the future in terms of past experience; to make, in short, effects precede and determine their causes. The cat's movements are in the main not merely reflex responses to stimuli. Rather they are throughout governed by the purpose of reaching the food. This involves some anticipation, however vague, of the goal. We may fairly suppose that, as the process is repeated, this anticipation becomes more definite, as also anticipation of the various steps of action by which the goal is reached (p. 195).

McDougall agrees with Koffka that the tests were not well adapted to the animals' capacities. Relating some experiments of his own in training an Airedale terrier to open a box by means of a lever and a latch, he emphasises as crucial the fact that the dog was always *trying* to open the box *in order to* obtain the biscuit he had seen placed therein—his behaviour was throughout purposive, even though he did not understand the mechanism at first. Nor when he had learned to open the box were his actions stereotyped and mechanical, as the Thorndike theory would require. On the contrary,

> not only did the movements not occur in the same order upon successive occasions, but the nature of the movements themselves continued to vary widely. Often he would begin by pressing the handle with his paw; and this was done sometimes with the right, sometimes

and more often with the left, forefoot. On finding it resist his pressure, he would usually run round at once to the latch; and this he would turn, sometimes with his nose, sometimes with his paw (p. 197).

The fullest and most important criticism of Thorndike's methods and conclusions is that published by Adams (1929). He went to great pains to repeat Thorndike's experiments as closely as possible, using replicas of three of Thorndike's puzzle boxes and testing a number of young cats therein. His other experiments with cats and his general conclusions as to the nature of the learning process we shall consider later (pp. 165–8); here we shall content ourselves with summarising his criticism of Thorndike, which falls roughly under three heads—technique, description, and interpretation.

In the matter of technique, Adams holds that the method of confining the cat in a box is bad for various reasons, partly because it prevents accurate observation of the cat's behaviour, partly because the mechanism is concealed and the connection between the act leading to release and the opening of the door obscure, but mainly because isolated confinement in close quarters may scare the cat and lead to panic behaviour. Adams emphasises the point, already made by Mills, that the "mad scramble" described by Thorndike as typical is certainly highly abnormal and indicates that the animal was not in a fit state for experimentation.

Secondly, Adams objects that the descriptions of behaviour were inadequate and contradictory. Whereas the meagre descriptions indicated a gradual elimination of useless movements, the time curves show for the most part an abrupt elimination, a sudden learning. Without details of behaviour it is difficult to interpret the time curves properly; for instance a long lag may simply be due to the cat remaining quiet and not trying to get out. Thorndike noticed, in the case of one or two cats which did not exhibit a fury of activity, that they paid more attention to what they were doing and solved their problem more rapidly that the active scramblers. Adams considers that these peaceable cats were the only ones in a fit state for experimentation.

Thirdly, on the question of interpretation, Adams maintains that Thorndike made a great mistake in comparing the behaviour of "naïve" cats with the behaviour of "sophisticated" men. Men, for instance, are familiar with action at a distance, with mechanical and other connections between action and effect; cats are not. Accordingly, to be able to infer the cat's mental processes from its behaviour in puzzle boxes it is necessary either to abstract from one's own experience of action at a distance, or to supply the cat with the opportunity of acquiring such experience. It appears from the experiments that those cats with some preliminary training, some experience of the results of their actions, do display the sudden drop in the time curve which may be considered indicative of ideation. But problems that appear very simple and very obvious to the human observer may be very difficult for the untrained cat.

Adams again calls attention to the fact recorded by Hobhouse and other observers that the method of solution was not stereotyped; in his own experiments, even after the cats had reduced their times to a consistent low level, there was much variation in the way any cat operated the mechanism from one trial to another. This fact alone seems to Adams to invalidate the simple "situation-response bond" theory. The situation does not call forth inevitably the same response; on the contrary, if it is a question of pulling a loop, the loop becomes a thing to be pulled, and the method of pulling it entirely secondary.

Adams devotes some attention to Thorndike's conception of "impulse" (see p. 151 above), and comes to the conclusion that "either this impulse consciousness is of the nature of purpose, intention or volition, in which case it is bound to be ideational; or it is a brand new psychological entity, with no known analogue in human experience, in which case it is superfluous" (p. 86). Thorndike had come to the conclusion that his cats had no power of inference; he stated that in his experience his cats never surveyed the situation and made up their minds what to do. Adams found that his cats did just this in the majority of cases. Usually the time spent in activity was small as compared with the amount spent in looking over the situation. The first solution was usually accidental, though one large cat never did anything but the successful act. Attention to the successful act seemed to facilitate repetition; the more deliberate and attentive the animal the sooner it acquired a high degree of efficiency. In general, Adams concluded, the learning could not be correctly described as the gradual elimination of useless movements. The process had this appearance only in the few cases where it was associated with excitement and consequent inattention. The total time taken was often irrelevant; it depended very much on whether the cat was trying to get out or was doing something else, *e.g.* washing itself. Adams found some evidence of learning by imitation; in some cases the attention of the cat was attracted to a particular spot or part of the cage by seeing another cat working the release mechanism, and this facilitated its solution. His final summing up about the Thorndike experiments and the Thorndike theory is severe:

> The data (descriptions) are inadequate. The conditions under which they were collected preclude valid conclusions from them, as to normal cat behaviour. Many of the inferences are erroneously drawn. The place of these experiments in the history of psychology and of science in general is honored and secure...but the influence of their conclusions continues to impede the progress of the field of science they inaugurated (p. 91).

We shall conclude this section by returning for a moment to the laws of learning proposed by Thorndike. In his latest work (1931) Thorndike comes to the conclusion that so far at least as human learning is concerned the law of exercise or law of frequency does not hold good.

> So far as I can now see [he writes], the repetition of a situation in and of itself has no selective power. If a certain state of affairs acts upon a man 10,000 times, he will, so far as any intrinsic action of the 10,000 repetitions is concerned, respond in the same way to the last thousand as to the first. The repetition of a situation may change a man as little as the repetition of a message over a wire changes the wire. In and of itself, it may teach him as little as the message teaches the switchboard. In particular, the more frequent connections are not selected by their greater frequency (p. 14).

Mere repetition of a connection has little or no power as a cause of learning; "belonging" is necessary, that is to say, some more or less "natural" relation between the situation and the response. The results of work on conditioned reflexes are apparently in opposition to this conclusion of Thorndike's and he is inclined to think that "conditioning" is not the fundamental basis of learning, but represents something different, something physiological rather than psychological. He still holds strongly to the law of effect as a fundamental law of learning. The laws of frequency and "recency" adopted by the behaviourists are adversely criticised by Peterson (1917), Kuo (1922), McDougall (1923, pp. 189–90), Washburn (1926, pp. 276–8), Higginson (1926), Helson (1927), Koffka (1928, pp. 175–6, 178–9), and others. The law of effect is generally admitted to have more validity, but much difference of opinion exists as to its interpretation.

III. CONNECTIONISM.

It will be recollected that Thorndike considered the connections between situation and response to be neural in nature, and learning to be due to facilitation of connections. Responses which are satisfying to the animal strengthen the connections involved; responses which are non-satisfying or lead to discomfort or annoyance leave behind them weakened connections. The theory is elaborated and linked up with the life processes of the individual neurones in his latest book (1931) in the following ingenious manner. He postulates that when the life processes of the neurone are going on well it continues whatever movement activity it is engaged in, extending for instance the tip of its axone fibre to make easy conduction possible across the synapse towards the next neurone. If, however, it is disturbed it tries other movements, until the interference with its normal functioning is removed.

> Now for the neurone's life processes of receiving and transmitting stimuli to go on well in a given state of affairs *is* the physiological fact that we mean when we say that the state of affairs is satisfying to the animal. For this conductive process in the neurones to be interfered with in a given state of affairs is the physiological fact that we mean when we say that the state of affairs is annoying. By the hypothesis, in the latter case the neurones move so as to hold some new spatial relations to neighbouring neurones. The neurones are, then, widening the gaps in those synapses conduction across which causes discomfort; are trying other spatial relations; and are maintaining those spatial relations—preserving the intimacy of those synapses—conduction across which causes satisfaction (pp. 57–8).

The law of effect thus works through and by means of the behaviour of the neurones, which manifest avoiding reactions strictly comparable to those of the Protozoa. "The simple avoiding reaction of the protozoa, inherited by the neurones of the brain, is the basis of the intelligence of man. The learning of an animal is an instinct of its neurones" (p. 59).

The hypothesis is hardly a happy one. The satisfyingness of an action depends upon its result; it seems quite illegitimate to identify this satisfiedness of the animal, which comes after and as a result of a particular chain of action, with a satisfiedness of the neurones which, if it exists, must be contemporaneous with the action and prior to its result. The theory is mainly interesting in showing to what lengths the physiological or analytical view can be pressed, and to what confusion it leads.

The view that learning is bound up with a wearing smooth of pathways in the central nervous system, and with establishing functional connections between paths originally separate, is one which is widely and somewhat uncritically accepted. It is, of course, definitely a physiological view, and like most purely physiological explanations much too crude and simple to cover the observed facts of behaviour. For example, it breaks down at once when confronted with the facts of perception. A dog learns very quickly to recognise its master, and shows characteristic behaviour on catching sight of him. The connectionist explanation is presumably somewhat after this fashion—the image of the master cast upon the dog's retina stimulates certain retinal cells, which have, by the process of learning, formed habitual connections through the cortex with the efferent neurones which effect the movements of welcome, etc. Passing over the difficulty, which Thorndike faces but fails to solve, of understanding how these habitual connections come to be formed, let us note one simple little fact. The image which elicits the response is not a fixed and invariable one, affecting always the same individual neurones; it may vary for example in size, according as the master is near or distant; it may be cast on different parts of the retina before fixation of the object is effected; more important still, the actual image may vary, according as the master is seen full face or sideways, or according as he is dressed. Yet, in all cases, an experienced dog will respond in its normal way to the varied retinal image. Obviously the individual retinal cell, with its individual connections, counts for nothing *per se*; any particular cell *A* may form almost any point in the retinal image; it is only its position relative to other points that gives it any significance, and that significance is a constantly changing one. In other words, what really matters in perception is the whole, the pattern, irrespective of what particular retinal cells are stimulated. If it be objected that perception takes place in the cortex, the same reasoning applies to any image presumed to be projected on to the cortex.

Take another case—I shall quote from Lashley (1929, 1930), who handles the point admirably—in human vision:

> with the eye fixed and a pattern moved across the field of the macula, the same reaction (*e.g.* naming the object) may be elicited at any one of a thousand points, no two of which involve excitation of exactly the same retinal cells. To say that a specific habit has been formed for each of the possible positions is preposterous, for the pattern may be one never before experienced. The alternative is that the response is determined by the proportions of the pattern and, within the limits of visual acuity, is independent of the particular cells excited.
>
> This means that, not only on the retina, but also in the central projection on the cortex, there is a constant flux of stimulation such that the same cells are rarely, if ever, twice excited by the same stimulus, yet a constant reaction is produced. The activity of the visual cortex must resemble that of one of the electric signs in which a pattern of letters passes rapidly across a stationary group of lamps. The structural pattern is fixed, but the functional pattern plays over it without limitation to specific elements (pp. 158–9).

A simple consideration, then, of some elementary facts about perception is sufficient to show the inherent improbability, not to say absurdity, of any strict connectionist explanation of either instinctive or learned responses, of either unconditioned or conditioned "reflexes."

There are also important experimental results which negative the connectionist theory, some of which we shall now consider. Wheeler (1929) calls attention to the following experiment carried out by Ewert on human subjects. They were trained to trace out with the right hand a star pattern in mirror-reverse; when proficiency was attained the left hand was then used; it was found that the proficiency of the left hand greatly exceeded that shown by the right hand on its first attempts.

> The learning was accomplished by the organism-as-a-whole [writes Wheeler], neural integrations were not confined at the outset to avenues between the eyes, the right hand and a limited section of the brain. Rather, the brain as a whole was involved, or at least nerve patterns permeating a large proportion of its structure. Still reacting as a total organism, the instant the observer commenced tracing a star with his left hand he already found the task practically learned, for the same central patterns continued to function (p. 303).

Lashley (1924 *a*) trained a white rat, blindfolded on the left eye, to distinguish between two lights of different intensity. When training was complete he transferred the blindfold from the left to the right eye; the animal continued to distinguish perfectly between the two lights. Another case reported by the same experimenter (1924 *b*) resembles the Ewert experiment, but is even more conclusive. Lashley trained a *Cebus* monkey to open a latch box with the right hand, the left hand and arm having been paralysed by operation. He then paralysed the right hand and arm likewise. After waiting till the right hand had recovered he again tested the animal; meanwhile the left hand had completely recovered and had been used in other manipulations. When confronted with the latched box the monkey first fumbled with the right hand in a clumsy manner and then shifted over suddenly to the left hand and opened the box. There was an almost perfect transfer of a habit learned by one hand to the hand which had been paralysed during the period of original training.

It is clear from these cases that the simple pathway theory is inadequate to account for the facts, that learning is something carried out by the nervous system acting as a whole.

The whole theory of nervous pathways, and their correlate, cerebral localisations, has been thrown into the melting-pot by the remarkable work of Lashley, which he has recently summarised and discussed in a book of fundamental importance (1929, 1930), to which we shall now turn.

When Lashley started his work he was, he tells us, under the influence of the theory of learning then current, according to which learning was due to random activities from which successful trains of action were selected and useless movements eliminated by a more or less mechanical process of "stamping-in" and "stamping-out." The neural equivalents of these mechanically formed habits were conceived to be reflex arcs, originally separate, but linked to one another in a certain order through the process of "conditioning."

> I began the study of cerebral function [he writes], with a definite bias toward such an interpretation of the learning problem. The original program of research looked toward the tracing of conditioned-reflex arcs through the cortex, as the spinal paths of simple reflexes seemed to have been traced through the cord. The experimental findings have never fitted into such a scheme. Rather, they have emphasised the unitary character of every habit, the impossibility of stating any learning as a concatenation of reflexes, and the participation of large masses of nervous tissue in the functions rather than the development of restricted conduction-paths (p. 14).

His experiments, carried out on rats, fall into two main series; in the first, the effect of cortical lesions on the learning capacity of the animal was tested; in the second, the animal was first of all trained, then operated upon, to determine what effect the lesion had upon the retention of the habit. In all cases, portions of the cerebral cortex were destroyed by means of thermocautery, the injury inflicted varying from case to case both in extent and in location, affecting from about 5 per cent. to about 80 per cent. of the cortical substance.

The rats were trained, before or after cerebral injury, in three simple enclosed mazes, in one open maze in which the animal runs along the edges of the vertically placed boards of which the maze is constructed, and in brightness discrimination.

As might be expected, the operated rats were slower to learn the maze habits than normal rats, and their capacity for retaining the newly learned habit was reduced. The extraordinary thing, however, is that Lashley found the deterioration in learning capacity to be roughly proportional to the *amount* of cortical destruction and to be not at all dependent on the *location* of the injury.

> The same retardation of learning is produced by equal amounts of destruction in any of the cyto-architectural fields. Hence the capacity to learn the maze is dependent upon the amount of functional cortical tissue and not upon its anatomical specialisation (p. 175).

This conclusion is very thoroughly established by Lashley, by a most careful mathematical study of the results and a full consideration of possible flaws and objections. He shows further that the interruption of the presumed association or projection paths, which was occasioned by many of the operations, produced little disturbance in behaviour, provided the cortical areas supplied by these paths remained in some functional connection with the rest of the nervous system. In particular he showed that destruction of the cortical areas which, on anatomical grounds, might be considered to be connected with the various senses concerned in maze-running, visual, olfactory, kinaesthetic and so on, had no specific effect upon learning. This is the less surprising because it had been shown by Watson (1907) that all the exteroceptors may be put out of action without seriously disturbing the capacity of the rat to learn a maze or to run one previously learned. Experiments by Lashley and Ball (1929) on sectioning the cervical cord show that very serious disturbance of both ascending and descending tracts does not destroy the maze-habit, though such interference naturally causes inco-ordination of movements.

> Animals which have learned the maze before the development of the motor incoordinations continue to traverse it, although the manner of progression may be almost completely altered. One drags himself through with his forepaws; another falls at every step but gets through by a series of lunges; a third rolls over completely in making each turn, yet manages to avoid rolling into a cul-de-sac and makes an errorless run (p. 137).

Another conclusion from this first set of experiments which deserves mention is that the more difficult the maze the greater is the retardation of learning caused by any given extent of lesion.

Omitting for the moment the effect of brain lesions on learning brightness discrimination we may consider the second series of experiments—those in which maze learning took place before operation. The results were similar to those obtained in the first series. The acquired habit is disturbed by lesions in any part of the cortex and the amount of disturbance is proportional to the amount of injury; it is also independent of the locus of injury. The effect on behaviour of lesions in different places is non-specific, and the retardation of re-learning is not referable to sensory defects.

The results with brightness-discrimination tests differ from those obtained in maze learning. The formation of the habit is not retarded by *any* cortical lesion up to 60 per cent. of the whole; operated animals may even be slightly superior to the controls. There is no definite relation in this case between extent of injury and amount of retardation of learning. If, however, the habit has been formed before operation, a different result is obtained. The habit is abolished by destruction of the occipital part of the cortex, but not by extensive injuries to other parts. On the other hand, the habit may be formed in the absence of the occipital cortex, some other part of the cortex, or, more properly, the whole residual cortex, taking on the function of the occipital part.

Somewhat similar results are obtained with a simple double-platform problem box.

Lashley discusses the difference between the results with the mazes and those with the other two tests, and points out that the latter are much simpler to learn than the mazes; they depend upon the formation of a simple association, which may be long delayed, but comes suddenly and is permanent once formed. The relative immunity of these habits from the effects of cerebral lesions he ascribes to their simplicity.

From his experiments as a whole Lashley draws the following important inferences:

> 1. The learning process and the retention of habits are not dependent upon any finely localised structural changes within the cerebral cortex. The results are incompatible with theories of learning by changes in synaptic structure, or with any theories which assume that particular neural integrations are dependent upon definite anatomical paths specialised for them. Integration cannot be expressed in terms of connections between specific neurons.
>
> 2. The contribution of the different parts of a specialised area or of the whole cortex, in the case of non-localised functions, is qualitatively the same. There is not a summation of diverse functions, but a non-specialised dynamic function of the tissue as a whole

3. Analysis of the maze habit indicates that its formation involves processes which are characteristic of intelligent behavior....

4. The mechanisms of integration are to be sought in the dynamic relations among the parts of the nervous system rather than in details of structural differentiation (p. 176).

The reader should bear in mind that only the bare outlines of Lashley's experiments and conclusions have been given in the above account, and he is recommended to study the book in detail. A very valuable criticism of connectionist views will be found on pp. 125–7, and 157 ff., particularly pp. 163–4 and 172–3. Further references of interest in connection with Lashley's views are Bartley and Perkins (1931), Freeman and Papez (1930), Gerard (1931), Hunter (1930 *a* and *b*), Simpson (1930), and Lashley himself (1931 *a* and *b*). Mention should also be made of MacCurdy's theory of "patterns" (1928), in which, in its special application to the central nervous system, he arrives independently of Lashley at very similar results. A recent restatement of the connectionist theory will be found in Holt (1931).

IV. CONATIONAL AND PERCEPTUAL FACTORS IN LEARNING.

I can best introduce this aspect of the problem—which presents some difficulty to the biologist who is unfamiliar with psychological concepts—by referring by way of illustration to some elementary experiments of my own with sticklebacks (Russell, 1931), in which conation and perception play a clear and simple rôle.

I set my sticklebacks a simple "detour" problem, analogous in general plan to many of the experiments carried out by Köhler with apes. A visually attractive object, namely a piece of worm, was placed at the bottom of a small glass jar in the aquarium; it could be reached only by the "roundabout way" of swimming up and in through the mouth of the jar. The behaviour of the fish was characteristic; at first they persisted in vain attempts to get at the food through the glass wall of the jar, biting vigorously at it time after time. With these attacks alternated periods of random swimming through the tank, in the course of which they sooner or later swam over the mouth, saw the worm at the bottom, went in and ate. After a very few chance successes of this kind, their behaviour changed; they alternated with the direct attack through the glass definite rises towards the rim of the jar, going in over the edge after a few of these tentative rises; as learning progressed entry was effected more rapidly, till in the end the fish sometimes went directly to the rim and entered; the number and duration of the futile direct attacks diminished very greatly, although the powerful direct attraction of the bait was difficult to overcome. The experiments further showed that the jar without food, presented either before or during the early period of training, was treated as an indifferent object, but very shortly after successful entry was achieved the jar itself, presented without food, became an object of attention, was nosed all over, and later entered.

Now the simplest and most accurate description of this behaviour is to say that the fish from the beginning *tried* to seize the food. Its efforts at first were badly directed, but they exhibited the objective signs of conative activity—persistence towards a goal, with varied effort. Chance success very soon brought a new direction into this general conative activity, and at the same time the jar became an object of "interest," as indicated objectively by the behaviour of the fish. That is to say, the jar was then for the first time actively *perceived*—there was a change in, a differentiation or organisation of, the sensory field.

This simple illustration will serve to indicate what is meant by conation and perception. Conation may be defined as an attempt on the part of the organism to change its state, or its relation to environment, in such a way that an end or goal is reached and the activity ceases. It may be from the first directed upon its object, as when a stickleback snaps up a piece of worm dropped into the aquarium; it may be wrongly directed or dispersed, as when the fish bites at the worm through the glass; it may become, through experience, correctly directed in a new way, as when the fish learns to take the way round, over the rim and in. This re-direction of the conative activity occurs *pari passu* with a change in the fish's perceptual world, whereby the way to the goal is, vaguely at first, perceived.

Thorndike's kittens show clearly this same change from ill-directed or, in this case, diffuse or random conative action, to well-directed conative activity, which is accompanied by a change in the perceptual situation, whereby certain elements of it, *e.g.* the releasing loop or catch, acquire significance, are attended to. So also do the animals studied by Hobhouse (see p. 154).

An important general theory which we shall now discuss is that all animal learning is based upon conative or goal activity, during the course of which learning there is a differentiation of the perceptual field, whereby the way to the goal becomes clear. On this view, all learning has incentive behind it, has a goal in front of it, is, in a word, *problem solving*.

The importance of the conational factor in learning is, as we have seen, clearly recognised by Hobhouse, Stout, Koffka, Adams, and particularly MacDougall. Hobhouse's "practical ideas," Stout's "continuity of interest," both imply conation. Lashley's description of the behaviour of maimed rats in a maze (see p. 163) clearly indicates the presence and power of conative activity.

We shall here consider in more detail Adams' formulation of his results, and the conclusions he draws from them; the fundamental contrast between the Thorndikean view and the conational will be brought out clearly thereby.

First a few words on some of Adams' other results, for in addition to repeating Thorndike's experiments with cats he added some new and interesting tests, which throw further light upon their capabilities. A number of experiments were carried out in which the cats had to move various types of lever in a horizontal plane in order to get food. Most of them failed to do this, but one or two succeeded. It has to be remembered that the lever is something unusual and does not look to the cat like a movable object. String-pulling tests were also tried with considerable success, some cats learning to haul in a horizontally lying string with food attached to the end; it was notable that one success was sufficient to bring about learning, and that minimum times were as a rule attained in the second or third trial. One cat solved the difficult problem of pulling up from the outside a string suspended inside a cage. Adams' description of this cat's behaviour is worth quoting.

> Tom walked all round the cage and looked over the whole situation from all sides, but did not once paw through the sides toward the liver. He frequently looked up the string to the place where it was tied, sometimes staring intently at it for some seconds. Finally, after one of these pauses, when he had been in the situation a total of two minutes and thirty seconds, he turned away suddenly from his intent stare, went to the right back corner and climbed to the top of the cage. He went directly to the centre, reached through the top and pawed up a loop of the string, but was unable to bring this through the wire (pp. 110–11).

After the cat had spent some minutes in unsuccessful effort, Adams untied the string and fixed it to a small stick lying on top of the cage, so that it could be easily pulled up. When Tom was readmitted he climbed up to the centre of the cage almost at once and pawed at the string where it was knotted to the stick.

> He then took the string in his teeth, moved backward about 15 cm., and sat down. Then with a sweeping motion of his right foreleg, using it as an arm, he took hold of the string with his paw as far in front of his mouth as he could reach, raising the liver perhaps 30 cm., stood on the portion of string gained thereby, and took a new and nearer hold with his teeth. Immediately, then, he turned his back on the reward, and jumped down from the cage, keeping the string in his teeth, and with the—to me—quite evident and confident anticipation that the liver would follow (p. 111).

Unfortunately he was balked of his well-earned piece of liver, which was knocked off as the skewer holding it caught in the mesh. Next day, however, he succeeded at once, taking the stick in his teeth and jumping down with string and liver complete. Adams considers that this behaviour indicates the presence of ideas. The successful results obtained in these and other tests, notably with "round-about" ways and delayed reaction experiments, suggested to Adams that his cats might not be so very different in capacity from the apes tested by Köhler (1925, 1927) and the Yerkes (1929). He tried therefore to repeat an experiment which succeeds with apes and monkeys, namely, to suspend a piece of food at such a height that it cannot be reached except by shoving a box beneath it and standing on the box. The obvious difficulty with cats is that they are not accustomed to using their paws to move large objects, nor in general do they regard large objects like boxes as movable at all. He therefore went to great trouble to train his cats to move a light box by means of pulling a string attached to it. This was a matter of considerable difficulty, but three animals were successfully trained in the art of moving boxes. Of these, one was successful in the main experiment, pulling a light box under the suspended meat, on her fourth trial. In her first trial also she succeeded, but almost certainly by chance; she pulled the box to the point directly under the meat, but without appearing to direct her movement thither. Her behaviour in the crucial experiment was as follows:

> The liver was suspended at a height of 85 cm., and the box was 50 cm., east of the point directly under the liver, with its long dimension at right angles to the direct line to that point. As soon as she was put in, Tabs climbed on the box and stretched repeatedly toward the liver. She shortly gave over this reaching, and walked around the room.

> After more than a minute of this, she stood up under the liver and reached toward it. Then she sat under it, and looked from it to the box and back again several times. Then she suddenly got up, ran to the box and started to pull it straight toward the liver. It was about 12 cm. away when she dropped the string, wandered off into a corner and sat down to wash without another glance at the liver. After more than a minute of this she suddenly paused and became rigid for a period of four or five seconds, in the posture of washing, with one hind leg sticking up at an angle of about 80 degrees from the horizontal, and with the liver throughout this period in her line of regard. Her ears were pricked forward and there was no relaxation from the awkward posture. At the end of this period of immobility, she abruptly got up and ran to the box, climbed on it and reached toward the liver. After several such futile efforts, she sat down and looked from the liver to the box, on which she was sitting, several times; then, again abruptly, she got down off the box, took hold of the nearer string, and pulled the box squarely under the liver, a distance of more than 30 cm., inasmuch as she had previously left the box with its nearest part about 12 cm. away from the point directly under the liver. She climbed on the box at once and got the liver. Time, 4 minutes 50 seconds (pp. 153–4).

Those who know cats will appreciate the vividness and accuracy of this description of cat behaviour, and the story is indeed a remarkable one. It exhibits clearly, as in a picture, the interaction of conation and perception.

At the end of his monograph Adams gives a generalised description of the behaviour shown by his cats in their various tests, in the following simple terms.

> 1. There is a cat. If one wished to be esoteric he might prefer to call it a proton-electron aggregate, which is thought by some to be much more scientific....It is not my task as a psychologist to study the behavior of protons and electrons, but that of certain of their aggregates which behave as units. The basis of differentiation between sciences seems to be their characteristic units of analysis, and that of psychology is the whole organism....2. There is something the cat wants. If you prefer, you may say instead, there is a special (described) physico-chemical disequilibrium in the proton-electron aggregate, and perhaps you can describe it. I prefer simply to say that the cat wants the thing, because that is more objective and more accurately descriptive....The thing wanted may be a state of affairs, such as freedom from constraint, and the thing may be wanted very much or very little. 3. There are other things too. Nearly everybody calls these the situation. The disposition of these other things may be such as to facilitate or to impede the cat's getting what it wants. 4. In one way or another, after certain movements with respect to the situation and the thing wanted, the cat gets what it wants....5. There is the cat again. 6. There is something the cat wants. 7. There is another situation, *more or less* like the first. This similarity to the earlier situation may be very great or very slight. 8. The cat gets what it wants more readily than it did before, that is, with greater economy of movement. This economy may be small or great. (Sometimes, of course, it is zero or there may be more movements. We are describing the more general case.) If the economy is small, it is often called trial and error learning. It might also be called a small insight. If the economy is very great, it may be called insight, or a big insight, or mental trial and error. 9. Series of events such as the preceding eight occur many times, some under experimental conditions, but most of them not. 10. Sometimes the situation is not *very* like any one earlier situation, but is more or less similar to a number of earlier ones. 11. Under these conditions the cat sometimes gets what it wants very suddenly, and with great economy of movement, after a period of no evident progress. 12. Thereafter in situations *very* similar to this last one the cat uniformly gets what it wants with great economy of movement. These last two phenomena are often called insight behavior (pp. 155–7).

This simplified description appears naïve and "unscientific," but it is actually an objective statement of the facts: such statement is of more value at the present time, in the undeveloped state of animal psychology, than any theoretical "explanation" or restatement in behaviouristic or physiological terms.

Adams' views on the nature of animal learning are in close accord, as he points out, with those of Tolman (1925, 1927, 1928), who regards learning as essentially problem solving, as implying action with reference to an end or goal. Before considering Tolman's views, however, it is essential that we devote some attention to the theory of "Gestalt" and to "insight," for both conceptions are having a profound influence upon the theory of animal learning, and it is impossible to discuss the more recent views without reference to them.

I shall assume in what follows that the reader is familiar with the classical work of Köhler on chimpanzees (1925, 1927), and I shall deal—or attempt to deal—rather with the theoretical groundwork.

The theory of Gestalt is primarily a theory of perception. It is the introduction into this field of the "principle of the whole," according to which any part of an organised state or process is determined or characterised not solely by its own intrinsic properties but by its *relations* to the whole. According to the Gestalt theory the perceptual field is essentially an organised whole, within which parts become differentiated out, but in such a way that their relations to the whole field constitute an essential element in their characterisation or definition. The perceptual field is not a summation or additive juxtaposition of separate elements; there are, strictly speaking, *no* separate elements to be added or juxtaposed; such elements as appear more or less separate can be adequately characterised only by their relations to the whole. Thus, to take the simplest case possible, a luminous point can be perceived only by its relation to, its contrast with, a darker ground. So too, the dimmer of two lights can be perceived as such only by comparison with the brighter. The idea of Gestalt is most fully developed and most easily understood in connection with visual perception.

It is necessary first of all to realise that visual perception is quite a different thing from the physiological action of physical or chemical stimuli upon the end-organ, the retina. If an "image" is cast upon the retina, this means, physiologically speaking, that a large number of adjacent visual elements are stimulated, and in different ways, according to the wave-length and intensity of the light rays impinging on each element. There is, however, strictly speaking, no image on the retina at all, except possibly to another observer, but merely a mosaic of differently stimulated elements, each of which is independent of, indifferent to, the others. If we see an image or pattern—as we do—this means that the indifferent mosaic is organised centrally, so that groups, or wholes, or Gestalten, patterns or images, are seen, and not a mosaic of unrelated light stimuli. Stimulation, as such, is completely unorganised; sensory organisation is necessary before perception is possible; and the animal responds, not to the raw physiological stimuli, which form an indifferent mosaic, but to its own perceptions, to the forms or patterns which it carves out of the sensory material. In visual perception, then, "the organism will respond to an objective constellation of millions of stimuli by developing, first of all, an organised field, many and perhaps the most essential properties of which have no physical partner among the single stimuli" (Köhler, 1930, p. 137). Action will in most cases be response to the visual field or situation as a whole, and if to parts of this field, then to these parts in their relation to the whole field.

> If...we say that in psychology the right formula is, *Constellation of stimuli—Organisation—Reaction to results of organisation*, such a statement fits the facts incomparably better than the usual one. The organism is not barren functionally; it is not a box containing conductors each with a separate function; it responds to a situation, first, by dynamical events peculiar to it *as a system* and, then, by behaviour which depends upon the results of that dynamical organisation and order (*ibid.* pp. 137–8).

When a male bird responds in characteristic manner to the sight of a female of the same species, it is hopelessly wrong to describe the female bird as the "stimulus" to the action, if by stimulus is meant, as should be meant, the physical action on the retina of the light rays reflected from the female. What the male really responds to is his own perception of the female, seen in her relation to her surroundings, and particularly in her relation to himself.

In dealing with an animal's responses we have then to do with reactions to perceived situations, and not as a rule with reactions to simple physical stimuli. Only in artificial experimental conditions is the animal subjected to pure or simple physical stimulation, and even then it is probable that the animal organises its sensory field in some way in conformity with its normal experiences, and reacts to the stimulus as representative of some normal perceptual situation. The fundamental assumption of the Gestalt theory is then that "instead of reacting to local stimuli by local and mutually independent events, the organism reacts to an actual *constellation* of stimuli by a total process which, as a functional whole, is its response to the whole situation" (*ibid.* p. 80).

In the development of the individual we find, by study of and inference from its behaviour, a gradual differentiation taking place within the perceptual field. Let us follow in this connection Koffka's description (1928) of the earliest perceptual experiences of the human infant. If a bright object appears in its field of vision the eyes move in an attempt to follow it; if the palm is touched the hand closes.

> If we wish to reconstruct the phenomenal counterpart of this objective behaviour we must consider the child's state as a *whole*. Consequently, we ought not to say that the child sees a luminous point; but rather that the child sees a *luminous point upon a relatively indifferent background*; or, in the case of touch, that pressure is felt in the hand, which before had been lacking in phenomenal distinction. Generally stated, *from an unlimited and ill-defined background there has arisen a limited and somewhat definite phenomenon, a quality* (p. 145).

The first phenomena of perception are, then, qualities or figures upon a ground, *i.e.* Gestalten or "configurations." Even the most primitive and earliest perceptions appear in the form of configurations. There is not present at first a confused mass of separate sensations, from which the infant learns by experience to select and to combine by association those that are of importance. If this

> theory of original chaos were correct, one would expect "simple" stimuli to be the first to arouse the reaction and interest of the child; because simple stimuli ought to be the ones first to be singled out from the chaos for association with one another. But all our experience runs counter to this assumption. It is not the stimuli the psychologist takes to be simple, because they correspond to his elementary sensations, that are most influential in the behaviour of a baby. The first differentiated reactions to sound are aroused by the human voice whose stimuli(and "sensations") are very complicated indeed. For instance, at the end of the first month the infant begins to scream when it hears another baby scream. Between the first and second month the infant reacts to the human voice with a smile, at first without differentiating between a friendly, neutral, or scolding voice. This differentiation occurs in the fourth or fifth month, when a smile is the reaction to friendly and inviting speech, while angry words evoke crying and general symptoms of discomfort (*ibid.* p. 147).

This principle of differentiation *within* a whole, as contrasted with the opposite principle of the combination of originally separate elements, is of course the same general principle that is applicable to all organic development (see, for example, Russell, 1930).

Just as in the early development of the child we get a slightly differentiated perceptual field, a simple figure-ground complex, so in the lower animals we find that the objects in their perceptual field are not clearly differentiated from one another, but are still to some extent merged or fused with the surroundings or "ground," so that the animal responds not to a clearly defined or separate object, but to the object as continuous with the "ground." The classical instance of this mode of response is of course the case described by Volkelt (1914). He made observations on a spider, probably a *Zilla*, which lies in wait for its prey in a tube adjacent to the web. When a fly is caught in the web the spider rushes out and attacks it. Volkelt found, however, that when he inserted a fly of the same species into the tube the spider did not attack it, but treated it as an enemy and fled from it. Volkelt deduced that the spider did not recognise the fly except in its relation to the web, that it perceived the complex "fly-web," but was unable to recognise the fly as a separate object. Certain criticisms have been made of Volkelt's experiment and conclusions (on which see Bierens de Haan, 1929) but in the main he seems to have been right.

A clearer case is afforded by the observations of Bierens de Haan on *Octopus vulgaris* (1926). This cuttlefish will instantly seize and eat a crab which is moving along the bottom, but Bierens de Haan found to his astonishment that it does not recognise a crab dangled in front of it, in close proximity to its eyes; it may even attempt to remove the crab by directing a jet of water upon it with its siphon. But as soon as the suspended crab is let down so that it can crawl on the bottom the octopus colours up at once and seizes it. The octopus does not recognise the crab in the unusual situation, but perceives it as an indifferent or annoying object. The experiment proves that the perception which

> gives rise to the instinctive actions of jumping and seizing the prey is of a complex character, namely, that of the crab making the special movements of swimming or creeping. Another

> complex, that of the crab sprawling on a string, does not give rise to the usual reaction. This shows, too, that the octopus is unable to detach the principal object from the new situation and to recognise in it the same object that was the centre of the other more usual situation (1929, p. 40).

Other examples, drawn from his own observations on monkeys, are given by Bierens de Haan (1929) which show that these animals react not to an isolated perceptual object, but to this object in its relations with the whole, or, more accurately, to the situation as a whole containing this object in certain relations to other objects.

One may refer here also to the numerous experiments which have been carried out of recent years to show that animals can be trained to respond to the *relation* between two stimuli rather than to the stimuli themselves. Thus Köhler (1915) found that chicks trained to react to the darker of a pair of greys, if given the choice of another pair, consisting of the darker of the first pair and one still darker, would in the majority of cases choose this new and darkest grey—they had learned to select, not a particular shade of grey, but the darker one of a pair. Many instances of this "relative choice" are now known (see Helson, 1927, Tolman, 1928, Wheeler, 1929, Perkins and Wheeler, 1930). The whole trend of this work, as of most modern work on the psychology of animals, is to show that animals do not normally respond to simple stimuli—simple in the physico-chemical sense—but to more or less complex whole situations, and if to parts of the whole situation then to these parts in their relation to the whole. This is the essence of the principle of Gestalt—response to elements in the perceptual field as parts of the *pattern* of the whole.

Before considering the relation of Gestalt theory to learning, it is well to point out that the principle of the whole is applied not only to the perceptual field but also to the overt action or behaviour which results from perception. Such actions are also "configurational," that is to say, show spatio-temporal unity; they are not essentially combinations of originally separate elements, but whole actions. R. H. Wheeler, whose enthusiastic exposition of the principle of the whole in his text-book of psychology (1929) is well worth reading, summarises the general position of the configurationists admirably as follows.

> We may propose the law that any reaction of the...organism-as-a-whole is a unified response made to a total situation of some kind and, if to a specific detail, always to that detail in relation to other details. We may call this total situation a stimulus-pattern or arrangement of stimuli....The reaction of the organism-as-a-whole is a pattern-reaction, or configurational response, and is not composed of isolated movements or a combination of discrete movements. Neither is it composed of discrete habits, instincts and wishes. It is an organised unit, and we call it a configurational response to emphasise the fact that it is, *first*, a response to a total pattern of stimuli, and *second*, that it is not a summation of discrete responses to discrete stimuli (1929, p. 77).

Applied to the theory of learning, the Gestalt conception leads us to consider the formation of associations as being, not the linking together by some mysterious bond of two separate experiences, but as the formation of a new pattern or configuration by a new organisation of the perceptual field, whereby new relations are established between the goal and the means of reaching the goal. Learning cannot begin until some sort of relation is established between what the animal perceives and the goal, until, in other words, the goal is perceived in its relation to the whole situation. This is not an easy conception to grasp, but it will I hope become clearer as we proceed. If we take in illustration the case of the stickleback described at the beginning of this section, it may be pointed out that learning does not begin until the shape of the jar, and its spatial relations to the position of the food, become significant, are attended to; the jar, from being an indifferent object, comes to take a prominent place in the fish's perceptual world; there is a new organisation of the perceptual field, in which there emerges an "association" between the jar, especially its shape, and the food.

A word as to what is meant by "insight," and we shall then be in a position to return to Tolman's theory of learning, after our long digression. The concept arose in connection with Köhler's classical work on apes. The reader will remember the famous instance in which Sultan suddenly discovered for himself the way to fit one stick into another, and immediately applied his newly acquired knowledge to practical ends by using the extended stick to rake in a banana which was previously out of his reach. This is an example of what Köhler calls "insight." The use of a box placed under a suspended dainty, or, more notably, the piling of one box on top of another to form an incredibly ricketty structure upon which the ape climbs to obtain the prize, also illustrates "insight." Insight solutions do not take place by trial and error, but by a sort of summing-up, or judgment, or appreciation of the situation, *before* the solution is put into operation. The solution, when it appears, is immediate and, within limits, adequate. The criterion of insight is therefore "the appearance of a complete solution with reference to the whole lay-out of the field" (Köhler, 1927, p. 190). Insight means, as McDougall (1930) rightly points out, the grasping of essential relations, such as temporal, spatial and causal, between the features or objects of the whole situation. Objects thus acquire functional value as means or tools in relation to the goal.

Tolman in his paper of 1927 discusses at some length the results of work on maze learning in rats—a type of investigation which has been pursued in America with much assiduity. He comes to the conclusion that maze learning must be envisaged as essentially problem solving. The learning of a maze is the discovery of the shortest and quickest or easiest way to a desired end or goal.

> The animal, by virtue of his discrimination and manipulation capacities, starts with a certain initial envisagement of the maze, out of which arises his set of initial exploratory impulses. It is the strength of his demands (drive) plus his capacities for discrimination and memory (*i.e.* his ability to discriminate short from long paths, to recognise blinds, etc.) which act as causal determiners to bring about a modification and improvement of this initial envisagement, together with a final selection of the true path only (p. 11).

The facts of maze learning are not to be explained on the hypothesis of a concatenation of conditioned reflexes, for a non-hungry rat, when he has learned a maze, will enter blinds in spite of the fact that they still present the negative cues. That maze running is not an unintelligent following of a routine path, due to a kinaesthetic habit, is shown particularly by the work of Higginson (1926). After training rats in a maze by means of 100 trials, Higginson opened a short cut to the goal.

> When the change was made only four of the nine rats ran into the blind and out again before turning into the newly opened door, and these four immediately shifted. And what is more significant, the remaining five "stopped suddenly and without interference ran the remainder of the maze correctly, thus dropping at once six feet from the previous pathway and turning in a different manner." These results, as Higginson insists, are inexplicable in the usual categories of kinaesthetic patterns and frequency and recency of performance (Tolman, 1928, p. 39).

Maze learning takes place only under the spur or incentive of a desired end; and it implies an improved knowledge of, or insight into, the position or relations of the end, an improved adjustment of the animal's behaviour with reference to the goal.

Tolman considers that both trial and error learning and insight learning essentially depend upon a new organisation or "re-Gestalting" of the perceptual field, whereby new meanings arise and new relations are found between the objects perceived. Thus, Thorndike's kittens have an initial "postulation" as to the position of the goal, and they use all normal means, such as scratching and biting and squeezing, to reach the goal. After learning, they have "a new improved postulation (insight) as to the position of the goal which expresses itself in the now acquired, especially strong propensity for pulling at anything like a hanging loop of string" (1928, p. 48).

Köhler's apes solve their problems in essentially the same way, but here the reorganisation of the perceptual situation, and the consequent solution, come without trial and error, suddenly, and prior to putting the solution into practice.

> The cat...learns only *after* she has actually pulled the loop and thus experienced its resultant success....In other words, we must assume that the learning arises in this type of case only when the relative values of the correct and wrong responses have actually been demonstrated through trying them out and obtaining actual experiences of their respective good and bad results. Consider, however, the case of insight learning. When the experiment is a crucial one, when, that is, it is crucially definitive of a so-called "insight" or non trial and error solution, the response which is finally chosen must *not* be one among the initial array of trials and errors. The ape must never before, in this sort of a situation, have used a stick. The virtue of this stick response must, then, in some way be discovered without, and before, actually trying it. The ape must "foresee" both its possibility and the goodness of its result. Herein, then, would seem to lie the peculiar essence of the primary or insight solution. In it, the new insight arises by "foresight" rather than as a mere by-product of acts after they have been performed (1928, pp. 48–9).

Both types of learning imply representation; in insight solutions, the act and its consequences are represented before the act is performed; in trial and error learning, representations arise in the course of learning. Such learning takes place only in so far as the good and bad results

> become in some way, rapidly or gradually, clearly or dimly, *represented* by the organism to himself at the moments before the acts leading to them are released. For only by

assuming such *representations* can we explain that the propensity towards the one act becomes re-enforced while those towards the other acts become weakened (p. 49).

This is Tolman's explanation of how the "law of effect" works. Insight or foresight learning is probably not limited to apes, but may occur in cats and rats as well. Thus, in Higginson's experiments the rats immediately took a short cut to the goal as soon as it was opened. Insight behaviour in rats or the sudden discovery of a novel solution is also described by Helson (1927), and is shown by some of Adams' cats.

But both in insight and in trial and error learning foresight arises—*before* experience in the former case, *through* experience in the latter.

After this general exposition of his standpoint, Tolman's final summing up of his theory of learning will be intelligible. It runs as follows:

> (1) All learning is to be said to arise out of an initial postulation of (insight into) the goal position, and to end in a new improved, or added to, postulation of (insight into) such goal position.
> (2) All learning may be said to involve the representation of the ends of acts at moments before their actual occurrence.
> (3) When these represented ends of acts are situations which, when actually present, lead at once (given the animal's innate and acquired endowment) to further appropriate responses, then the propensities towards the acts leading to those ends will become strengthened.
> (4) When, on the other hand, these represented ends of acts are situations which when actually present lead only to negative or avoidance responses, then the propensities towards the acts leading to those ends will become weakened.
> (5) The higher the animal, the fewer the number of experiences of an act which are probably necessary to achieve such representations of its end, and the clearer and more accurate such representations themselves probably are.
> (6) The higher the animal, the more it would seem that these representations can be played with and manipulated; the more the animal can mentally add and subtract the acts to produce new representations; the more, in short, he can achieve "foresight," as opposed to mere trial and error solutions (1928, p. 51).

A very full exposition of the Gestalt theory of learning is given by Wheeler (1929) in his book already referred to. According to Wheeler the animal is a member of the configuration in which it moves, and unless it perceives the goal in the stimulus pattern as a whole the goal is not established. The guiding or directing of activity with reference to the goal cannot take place until there is established in the animal's perceptual field a pattern in which the goal appears. Association therefore arises *with* learning, not prior to learning. If the goal is vague or not appreciated as such by the animal—as may easily happen in badly conceived experiments—the animal may not perceive it, but seek other goals of its own, or exhibit purely random trial and error movements, achieving success purely by chance. Learning begins when objects are seen in their relations to the goal, that is to say, when the goal and the related objects become differentiated out *in connection with one another*, or "associated" with one another. Association then is not the linking together of two separate percepts already existing as such in isolation from one another; on their appearance they are already linked, and are perceived *as* linked, *as* associated. Learning is easiest if there is a meaningful link between the associated objects, if the problem to be solved is a "sensible" one, and not one depending upon purely arbitrary associations.

Wheeler, like Tolman, considers learning to be essentially goal activity, and to depend upon an organisation or differentiation of the perceptual field. Instead of "conation," however, he speaks of "tensions" or "stresses" in the organism which tend to be released or relieved in the direction of least action, just as they would in an inorganic system.

The importance of conation, regarded as a fundamental attribute of living things quite different from anything inorganic, has always been strongly stressed by McDougall, with whose paper on "Insight and Foresight" (1931) we may conveniently conclude this cursory survey. In this paper McDougall describes experiments carried out with a Bornean monkey (*Macacus nemestrinus*), two raccoons, the rats used in his Lamarckian experiments (McDougall, 1927 *a*, 1930), and a mason wasp, from which he concludes that these animals exhibit not only insight but foresight as well. To take some of his observations—the monkey was kept on a light chain attached to the foot of a tree and to a belt round her waist; the chain was looped once round a stake so placed as to prevent her reaching a banana laid on the lawn; after some vain attempts to unwind the loop she seized it in both hands and tried to lift it off the stake, succeeding in this after several attempts.

In subsequent tests she succeeded more rapidly and reduced her time from several minutes to twenty seconds. McDougall holds that this experiment shows the monkey to have acted not only with insight into the relation of the chain to the stake but also with foresight of the result of lifting off the loop. It is noteworthy that to deal with the loop she turned her back upon the bait; there could not therefore be present in her visual field the complete configuration or pattern comprising the goal to be reached. A somewhat similar experiment succeeded also with one of the raccoons; in this case the stake was light and fitted loosely into a hole in the ground; the raccoon quickly learned to pull up the stake by digging round it. In a simpler experiment where the chain was simply passed round the post, the raccoon often backed towards the post, keeping her eyes fixed on the bait, and reached out backwards with her forepaw to grasp the post and then pass round it. "Dum's action," writes McDougall, "in reaching out backwards towards the post as she backed towards it seems to me an interesting bit of evidence of action directed towards an object not in the perceptual field, and, therefore, forming no part of the visual or perceptual configuration" (p. 260).

Many instances of foresight, of modification of behaviour in anticipation of consequences, were noticed by McDougall in the course of his Lamarckian experiments with rats.

He considers indeed that *all* conative activity implies foresight, even though it be of the vaguest kind. For instance:

> A rat, placed in a water-maze furnished with a single route of escape from the water, swims perpetually to and fro until he finds the place of exit. This swimming to and fro might plausibly be described as random locomotion. Yet repeated observation of this

> behavior in hundreds of instances convinces me that, even when a rat is taken from the nest box in which it has been born and bred and is immersed in the water for the first time in its life, its swimming is not utterly random, not entirely blind; even on this occasion the rat is seeking a way out, is looking for a way of escape. This seems to me a typical form of primitive goal-seeking behavior prior to all experience of the goal. The rat cannot be supposed to form any picture or representation of any particular way out. Yet he is not merely swimming to and fro; rather he explores the tank, not systematically, yet in general effectively; and, if the experiment is varied by providing no route of escape from the water, the great majority of rats, after thoroughly exploring the surface of the water and its boundaries, will extend their search for an outlet by diving to the bottom of the tank and there continuing their exploration....There is here, I suggest, that vague form of anticipation or foresight which characterises most forms of purely instinctive behavior. The foresight is utterly vague, involves no definite cognitive content; yet the behavior is forward-looking; there is a gap to be filled, and, as soon as the appropriate object is found, a hole in the containing wall, or a gangway leading out of the water, the rat accepts it as filling the gap, and guides his movements by reference to the discovered route of escape (pp. 263–4).

In general, "all conative activity, even in its simplest forms, must be supposed to be intrinsically forward-looking and anticipatory; and all that repeated experience of similar situations does is to fill in and define the vague gap which the conative activity seeks to fill" (p. 264).

In an earlier paper (1927 *b*) McDougall had shown that rats learn to pull in strings to which food is attached, and that their action is guided by anticipation of success. They do not pull up the string if they are not hungry; if some inedible object is substituted for the food the rat will haul up the string a few times and then lose interest in it.

> The action of hauling in the string is therefore not merely the expression of a habit (no matter how many times it may have been repeated); it is the expression of a conative urge, an urge to obtain the food, guided by anticipation of success, by insight into the relation of the string to the food and by foresight of the effect of hauling on the string (1931, p. 265).

As in so many other cases of manipulative learning, the movements are not stereotyped; the rat may use his paws or his teeth, aided by the muscles of his head and neck.

Observations on the nest building of a mason wasp and its ability to repair damage to the structure lead McDougall to reaffirm his conviction, expressed in 1923, that instinctive actions are not entirely blind, but involve a modicum of insight and foresight, some dim representation, not indeed of the biological end of the instinctive action, but of each immediate step in the chain. The instances of repair to the cells after human interference

> show first that the whole activity of cell-building has conative continuity; secondly, that this conation is satisfied only by the completion of an intact cell; thirdly, that, when the wall of the cell is partially destroyed, the wasp appreciates the fact, and, interrupting the normal course of building, repairs the breach; fourthly, that she has insight into the general relations of the cell, the egg and the spiders with which she stocks the cell, an insight limited it is true, yet generally sufficient to enable her to rectify any gross dis-

turbance of the normal course of the whole process under the guidance of foresight of the completed whole (p. 270).

While then McDougall agrees with the Gestalt school in laying stress upon insight, and to some extent upon the re-organisation of the perceptual field in learning, he goes much farther than they do in his treatment of conation. The Gestalt psychologists reduce conation to a dynamical tendency within the organic system towards "closure"—towards the establishment of an equilibrium, just as happens in any unstable inorganic system. McDougall maintains on the contrary that conation is essentially a psychical activity in which there is always reference, however vague, to the future. "The definite foresight that guides action when the circumstances have become familiar can be conceived only as developing out of the vague well-nigh contentless anticipation of the first instinctive striving" (p. 264). He foresees that the Gestalt school will go forward "to the full recognition both of the *conative impulse* and of the *guiding foresight*, without which *insight* alone can accomplish nothing" (p. 271). A position in some ways intermediate between those of McDougall and the Gestalt school is that taken up by Gengerelli (1930), who on the basis of maze experiments with rats comes to the conclusion that a general law of learning can be formulated in the following terms: "The organism, under the stress of a need, tends in consequence of repetition, to relieve that need by the process of least effort" (p. 228).

V. SUMMARY.

1. Thorndike's theory of animal learning, which is of considerable historical importance, has been subjected since its first appearance in 1898 to much criticism especially from the psychological point of view. A brief account is given of these criticisms, with special reference to those which emphasise the conational element in animal learning. Adams' recent repetition of the Thorndike experiments, and the conclusions adverse to Thorndike's theory which he draws therefrom, are treated in some detail.

2. According to Thorndike, the bonds or connections between situation and response are neural in nature. The deficiencies of the theory of "connectionism" as applied to the facts of perception are brought out, and experimental evidence adduced that learning is not to be explained as due to the formation of neural connections, but involves the action of the nervous system as a whole. The important work of Lashley, which throws doubt upon the whole theory of neural pathways and upon the existence of cerebral localisations, is considered in outline.

3. An account is given of certain modern views, especially those of Adams, Tolman and McDougall, which lay stress upon the conational aspect of learning, and upon the changes in the perceptual field which accompany learning. The "principle of the whole" as applied by the Gestalt school both to perception and to action is discussed in this connection, together with the part played in learning by "foresight" and "insight."

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THE PHYSIOLOGY OF SUCCULENT PLANTS

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I. INTRODUCTION.

AMONG the diversities of form and structure met with in the plant kingdom, the succulent habit seems to stand out in peculiar prominence. The general idea conveyed by the term "succulent," a certain fleshiness or juiciness of the plant or plant organ, is at first sight clear enough. When, however, we begin to analyse it and attempt a scientific definition we are at once beset with difficulties.

The succulent habit is not definable by any single morphological or anatomical characteristic. Succulents themselves are not wholly succulent: the term may be applicable to the stem or the leaf or to both, or even to certain tissues of the stem or leaf, rarely if ever to the root. The characteristics of the habit include a collection of features most of which are commonly but not universally present. The term succulent is often used in a wider sense to imply a certain juiciness of plant organs (*e.g.* succulent fruits) associated with a high water content of the tissues (cf. Pearsall and Ewing, 1929, p. 27). Moreover, some degree of succulence may be a feature of plants which are not usually regarded as succulents.

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It has long been held, however, that with succulency as an attribute of succulent plants in the narrow sense are associated certain peculiarities of metabolism, the latter being directly or indirectly a result of the succulent "make-up."

This review aims at an analysis of the available data to see how far the physiological features attributed to succulents are truly general characteristics, how far they are peculiar to succulent plants, and to what extent they are caused by characters of form and structure usually associated with the succulent habit.

The explanations of the peculiar metabolism of succulents have mostly been based on the morphological and anatomical characters of the group. A relatively small number of stomata and extreme cutinisation of the epidermis, together with massiveness of the internal tissues and a poor development of the air-space system, which is generally believed to be characteristic of succulents, have been put forward as features which retard gaseous interchange. In consequence, it is held, of the resulting inefficiency of the oxygen supply, the respiratory processes end in the formation of organic acids, instead of being complete to carbon dioxide and water. The structural features referred to affect the water relations, and it is mainly in this connection that they have been studied. The first section is therefore devoted to a brief consideration of the water relations of succulent plants.

II. THE WATER-RELATIONS OF SUCCULENT PLANTS.

(1) *Characters of the external tissues influencing the water relations.*

Many succulent xerophytes have a thick cuticle and relatively little water loss (de Bary, 1884; Delf, 1912; Haberlandt, 1914 and others). Aubert (1892*a*) on the other hand states that many Crassulaceae and species of *Mesembryanthemum* have a thin cuticle. Many halophytes, and succulent epiphytes, according to Holtermann (1907) and Delf (1912), also show but little development of cuticle. It is obvious therefore that varying degrees of cutinisation are found in different succulent types.

Hairs, whether glandular or protective, are usually absent, but there are a few examples of hairy succulent plants, *e.g. Sedum villosum*, *Sempervivum arachnoideum*, *Evolvulus alsinoides* and some species of *Mesembryanthemum*.

The experiments of Burgerstein (1904) and Delf (1912) point to the conclusion that whereas in those succulents with a thick cuticle, the cuticular transpiration is almost negligible, in succulent halophytes and in other succulents with a thin cuticle, the cuticular transpiration may be of appreciable magnitude.

According to Delf (*loc. cit.*) and Czech (quoted by Delf), succulents have fewer stomata than mesophytes, and also succulents from dry regions, such as *Mesembryanthemum* and *Sedum*, have considerably fewer stomata than those, such as *Salicornia*, from marshes. In the halophytes, and in *Mesembryanthemum* and many Crassulaceae, the stomata have been shown to close at the earliest stages of wilting.

(2) *The transpiration of succulents.*

The data on the transpiration of succulents, especially desert succulents, are rather scanty. Aubert (1892*a*) concluded that for equal surfaces many Crassulaceae and species of *Mesembryanthemum* which have a thin cuticle transpire more freely than many mesophytes with a thick cuticle (*e.g. Hedera helix*, *Picea excelsa*); for equal fresh weights, however, the succulent types transpire less than these. The Cactaceae transpire less than any plants examined, whether reckoned on equal surfaces or equal fresh weights. Delf (1912) has also confirmed the high transpiration rate of the British Crassulaceae and of those species of *Mesembryanthemum* which have become acclimatised, as compared to ordinary mesophytes, although the latter usually possess the more numerous stomata. Livingston (1907) and E. B. Shreve (1915, 1926) have shown that the cacti not only differ in their rate of transpiration from other desert succulents but also show a curious anomaly in the diurnal course of their transpiration. Contrary to what is found in other plants, the relative transpiration[1] of cacti is lower in the daytime, and higher during the night. This anomaly is probably due to the closure by day and the opening by night of the stomata.

The transpiration of halophytes has been investigated by Stahl (1894), Rosenberg (1897), Delf (1912), and others. These investigators found that many typical halophytes have a high rate of transpiration per unit area of surface, which is comparable with, or even higher than, that of mesophytes. Holtermann (1907) and Kamerling (1912) also showed that tropical strand plants have a very high rate of transpiration; in this case rapid loss of water was only experienced for 2 or 3 hours daily when the water stored in the tissues was largely utilised.

It appears, therefore, that halophytic succulents as a class are capable of very rapid transpiration rates, and that other succulents vary very much amongst themselves in this respect.

(3) *The osmotic pressure of succulents.*

It is a well-established fact that the osmotic pressure of desert succulents is low as compared with other types of plants which have been investigated. McDougal (1912), Delf (1915), Harris and Lawrence (1917) and Maximov (1928), have shown that a wide range of succulent plants have a very low osmotic pressure, seldom exceeding 10 atmospheres. These observations are in direct disagreement with the older view that succulent plants maintain a high osmotic pressure in their cell sap by the formation of organic acids and the production of mucilage, an idea which partly owes its origin to the work of Aubert (1892*a*). In their osmotic pressures the desert succulents thus differ markedly from the typical xerophytes, which have been shown by Fitting (1911) to have very high osmotic pressures. They also differ from the halophytic succulents which, like the typical xerophytes, have high osmotic pressures. The halophytes can increase their osmotic pressures when they have to absorb water from highly concentrated solutions even to 100 or more atmospheres (Fitting, 1911).

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[1] *I.e.* the ratio of the rate of transpiration to the rate of evaporation from a water surface of equal area under similar conditions.

(4) *Water absorption, and the water reserve.*

Succulents differ from most other plants in having a comparatively large water reserve, this reserve being replenished by absorption during the rainy periods, depletion taking place during the ensuing drought (McDougal and Spalding, 1910; McDougal, 1912; Bews and Aitken, 1925 and others). In some of the desert succulents new rootlets are formed at the beginning of the rainy season. The rootlets are deciduous, being shed as soon as the dry season sets in (cf. Cannon, 1912). The superficial root system of succulents enables them to absorb water even though the supply is scanty and does not penetrate to any appreciable depth. On the other hand Cannon found a few with deep roots.

In addition to possessing a large water reserve, succulents often show a marked conservation of the water supply, the younger leaves in time of drought being kept continually turgid at the expense of the older. Interesting in this connection are the observations of Pringsheim (1906), which showed that with very few exceptions the lower leaves had a lower osmotic pressure than the upper. The osmotic pressure was greatest at the growing point, falling off quickly and remaining fairly constant for the adult leaves.

The proportion of their water content, the loss of which succulents can survive, is also generally much greater than in other plants. There are xerophytes, however, which are capable of withstanding large changes in their water content without injury: thus Thoday (1921) found that in species of *Passerina* half the original water content could be lost without permanent injury resulting. In this case the actual water content could sink as low as 26 per cent. It is doubtful if the water content of succulents ever falls as low as this, for loss of dry substance also occurs. The data of Bews and Aitken (1925) for *Portulacaria afra* provide evidence pointing in this direction.

(5) *Summary.*

An analysis of the water relations of succulents as a class demonstrates the difference between desert succulents and halophytic succulents. The former are characterised by low, the latter by high osmotic pressures. The halophytes show high rates of transpiration; the xerophytic succulents fall into two classes, (*a*) those like the Crassulaceae, Mesembryantheae, and others which show comparatively high rates of transpiration per unit area of surface, and (*b*) those like the Cactaceae which show a low transpiration rate. In the Cactaceae it is generally higher during the night than during the day.

Owing to the small surface exposed, however, the rate of water loss per cent. of the original fresh weight is low for succulents, and their water reserve enables most of them to live without any external water supply for considerable periods. The latter capacity does not seem to be so marked for the halophytic succulents.

III. THEORIES OF THE ORIGIN OF THE SUCCULENT HABIT.

(1) *Earlier theories.*

Several attempts have been made to explain the origin of the succulent habit, most of them based on investigations which dealt with a few aspects of this habit only, or were confined to some special class of succulents. All these theories, and indeed some of the later ones also, insist on some aspect or aspects of the environment as being directly concerned.

Vesque (1883–4) was of the opinion that succulence was caused indirectly by a heating of the soil. This, he found, resulted in a higher osmotic pressure in the roots, and he inferred more vigorous absorption of water by the root system, which in turn enabled succulent plants to acquire large water reservoirs and considerable volume. For halophytes the supply of nutrient solution is alternately strong and weak, and Vesque suggested that increase of sap concentration when the solution is strong is followed by distension of the cells when the solution is weak. These suggestions appear to be, in essence, attempts at a mechanical explanation of succulence. Vesque seems to imply that vigorous root pressure leads to distension of the cells in the shoot system, but if water were pumped into the shoot system the pumping pressure would not cause distension of the cells, but a flooding of the air spaces. Moreover, the osmotic pressure, and therefore the suction pressure, in non-halophytic succulents is generally low, so that the considerations on which Vesque's views were based are unsound.

Warming's view (1909, pp. 124 and 371–2) is one of adaptation to environment. He does not, however, confine himself to one aspect of the environment and, while attaching value to the observations of Vesque, quotes also the experiments of Vöchting and Goebel, which showed that the peculiar shapes of leaf-like Cactaceae are mainly induced by light, and of Lesage, who showed that certain dimorphic halophytes are more succulent when grown in a halophytic environment than when grown in soil free from salt.

Warming's view is essentially the same as that of Henslow (1893), who maintained that succulence "is one of the direct results of intense heat (probably influenced by the presence of salts in the soil) inducing the formation of a thick cuticle, which in turn involves the retention of water, and the development of succulent aquiferous tissue."

(2) *The pentosan theory.*

The origin of xerophytism and succulence was to some extent discussed by McDougal and Spalding (1910) mainly in relation to the Cactaceae. According to the views then advanced, the reduction of the leaves, production of spines and induration of the surface may be regarded as the more primitive or initial modifications which arose under desert conditions, and the formation of tissue accommodating a large water reserve as a secondary or subsequent change of a more highly specialised character. Succulency is manifested by many plants in which the primitive xerophytic modifications have not been extensive, and a water reserve is carried in roots, stems and special organs. They inferred therefore that succulency is not the direct result of the "simple causes" leading to xerophytism. More recently McDougal has altered his views to some extent. His hydration and imbibition measurements led him to believe that the protoplasm of plants was "in the main" composed of pentosans, since the two substances showed a similarity in their hydration properties. Since succulence implies an increased storage of water, attention was turned to the pentosans as a possible explanation. Spoehr (1919), one of McDougal's co-workers, found that the succulents examined contained a certain amount of pentosans, and that dry conditions and high temperature favoured the formation of these substances. This is stated to mean a conversion of hexose polysaccharides with but little hydration capacity into pentosans which have a large coefficient of imbibition. According to McDougal and Spoehr (1918), this change, accompanied or followed by an enlargement of cells, results in succulence. Under other conditions, however, low water content and high temperature cause the formation of anhydrides such as cellulose: or at least such action is increased or accelerated. They suggest that such a use of its carbohydrates by the plant results in a limited growth, particularly where the effects of aridity would be greatest, and the surface becomes hard and indurated. Indeed they state that these two separate types of transformation might take place in the same plant in different cells. They conclude (*loc. cit.* p. 242) that "succulency results from the conversion of polysaccharides to pentosans or mucilages, and xerophytism, from a conversion of the polysaccharides into the anhydrides or wall material, both transformations being induced by a depleted or lessened supply of water in the cells." Richards, another of McDougal's co-workers, discovered (1918*a*) that in the case of *Castilleja latifolia*, *Ericameria ericoides* and *Erigeron glaucum* a mesophytic and a succulent form exist. The thin leaves of the mesophytic form show an acidity double that of the fleshy type, and have a greater percentage dry weight. The fleshy leaves, both when fresh and in a dried condition, like the Platyopuntias, swell more in alkaline than in acid solutions, in contrast to the thin leaves which swell more in acid solution.

We may summarise the view at present held by these authors as follows: Succulence arises by a conversion of carbohydrates with a small imbibition capacity into pentosans with a capacity to hold and absorb large quantities of water; these changes are caused by low water content and high temperature, and take place most readily in plants which have a type of metabolism leading to large acid residues. Whatever causal value is attributed to the action of soil salts or of acid conditions will rest upon their part in the conversion of polysaccharides to pentosans.

(3) *Critical consideration of the pentosan theory.*

McDougal, Richards and Spoehr (1919, p. 415), speaking of pentosans, state: "These substances probably are always present in some proportion in cells, and their occurrence is therefore not significant. Any action or condition which brings about a notable increase in their proportion in the cell, would have most important consequences however." If succulence is caused by the formation of large quantities of mucilage, which action, according to the above authors, is irreversible, we should at least expect the succulents as a class to contain vastly greater amounts of pentosan than non-succulent plants. The available figures for the pentosan content of succulents and some for non-succulents are collected together in Table I.

Some investigators have distinguished between: (i) Water-soluble pentosans (extractable by hot water), which are probably in solution in the cell-sap. (ii) Those soluble in 1 per cent. HCl, which are probably loosely combined pentosans not in solution in the cell-sap, since they are only extracted by water after hydrolysis. (iii) Those which are hydrolysed by 12 per cent. HCl, these being in all probability part of the wall structure.

Table I. *Pentosan content.*

A. SUCCULENTS.

Authority and material used		Pentosan extractable by 1 % HCl (including water-soluble) % dry wt.	Total pentosan % dry wt.	Total pentosan % fresh wt.	Remarks
SPOEHR (1919)					
Opuntia phaeacantha:					
	June 10	14·81	—	—	For seasonal variations
	July 5	9·04	—	—	
	July 31	4·14	—	—	
	Sept. 20	8·86	—	—	
	Oct. 27	10·47	—	—	
	Nov. 15	11·35	—	—	
	Dec. 20	10·10	—	—	
	Old joint	6·70	—	—	Same day
	Young joint	9·55	—	—	
	5 p.m.	8·34	—	—	For diurnal changes
	7.30 a.m.	8·22	—	—	
	5 p.m.	8·55	—	—	
	A	1·10	—	—	A, control; B, after 44 days in dark at 20° C. kept dry; C, as B but kept moist
	B	1·61	—	—	
	C	0·96	—	—	
O. phaeacantha		1·13	—	—	Same time, grown under similar conditions
O. versicolor		7·26	—	—	
BEWS and VANDERPLANK (1930)					
Portulacaria afra:					
Leaves	May 9	—	4·6	0·598	For seasonal changes
	June 19	—	5·73	0·997	
	July 30	—	6·40	1·39	
	Aug. 17	—	4·95	0·723	
	Oct. 3	—	5·15	0·644	
	6.30 a.m.	—	4·6	—	May 9–10; for diurnal changes
	5 p.m.	—	4·93	—	
	6.30 a.m.	—	4·69	—	
	S.	—	3·50	0·399	Same tree: S., south (shaded) side; N., north (insolated) side (S. hemisphere)
	N.	—	4·60	0·598	
Stem	May 18	—	4·14	—	For seasonal changes in woody stem. July 19, weather dry; July 30, very dry; Oct. 3, new leaves produced
	June 19	—	4·28	—	
	July 30	—	4·70	—	
	Aug. 17	—	4·24	—	
	Oct. 3	—	4·47	—	

Table I (*continued*).

B. OTHER PLANTS.

Pentosan per cent. dry weight.

		Water-soluble	Extractable by 1 % HCl (including water-soluble)	Total	
DAVIS, DAISH and SAWYER (1916)					
Mangold leaves	Aug. 26	—	—	5·19– 5·96	Determinations at 2-hourly intervals throughout the day
	Sept. 10	—	—	4·42– 5·90	
	Oct. 12	—	—	6·21– 6·77	
Mangold petioles	Aug. 26	—	—	9·8 –10·6	
DOYLE and CLINCH (1926*a* and *b*)					
Larix leptolepis	May	0·79	3·81	3·81	
L. europaea	May	0·66	2·90	2·90	
	Oct.	0·36	3·38	3·93	
Beech	June	0·63	8·94	10·24	
Sycamore	June	0·86	4·33	6·54	
Abies pectinata	I	—	5·14	5·84	Comparison between two successive winters, I and II
	II	—	5·15	6·09	
Austrian pine	I	—	5·93	7·36	
	II	—	5·93	7·27	
Sitka spruce	I	—	4·17	5·34	
	II	—	4·10	5·26	
Douglas fir	I	—	3·57	4·06	
	II	—	4·85	5·55	
Cupressus	Oct.	1·69	—	—	
	Nov.	2·30	7·10	8·07	
	Jan.	5·06	6·31	7·39	
Tsuga	Nov.	0·45	—	—	
Austrian pine	Nov.	0·55	6·05	7·45	
BEWS and VANDERPLANK (1930)					
Hypoxis rooperi:					
Leaves	Mar. 23	—	1·28	5·84	Green
	May 1	—	—	6·64	Dying
	May 19	—	2·06	6·66	Dead
	Oct. 3	—	5·08	9·51	Spring leaves
Corms	Mar. 23	—	c. 1·86	3·06	Leaves green
	May 1	—	—	3·25	Leaves dying
	June 14	—	—	3·26	Resting
	Aug. 13	—	—	3·87	
	Aug. 28	—	—	3·10	Buds developing
	Oct. 3	—	—	3·19	Spring activity
VERHULST, PETERSON and FRED (1923)					
Zea maïs	Plant	—	—	15·4	
ANDERSON and KULP (1922)					
Maize pollen		—	—	9·6	
ROSA (1921)					
Cabbage	I	—	—	3·0–4·6	In greenhouse, under different moisture conditions: I, optimum (tender); II, medium; III, minimum (hardy)
	II	—	—	3·0–5·26	
	III	—	—	3·3–8·20	
Tomato	I	—	—	3·0–7·1	
	II	—	—	3·7–6·1	
	III	—	—	2·5–6·1	
Lettuce	I	—	—	2·12	
	III	—	—	4·31	
Kale	Oct. 7	—	—	3·93	Garden plants in autumn
	Oct. 20	—	—	4·89	
	Nov. 3	—	—	3·93	
	Nov. 10	—	—	4·95	
	Nov. 18	—	—	6·48	
Celery	Oct. 7	—	—	4·42	
	Oct. 20	—	—	4·26	
	Nov. 3	—	—	4·44	
	Nov. 10	—	—	5·58	

Table I (*continued*).

Pentosan per cent. fresh weight.

		Water-soluble	Extractable by 1 % HCl (including water-soluble)	Total	
Rosa (1921)					
Cabbage leaves	March	0·075	—	0·215	Wet grown greenhouse plants
		0·292	—	0·423	Dry grown greenhouse plants
		0·091	—	0·207	Greenhouse; not hardened
		0·408	—	0·530	Hardened in cold frame 2 weeks
		0·550	—	0·776	Hardened in cold frame 3 weeks
Tomato	May	0·070	—	0·693	Wet grown, in greenhouse
		0·071	—	0·720	Dry grown, in greenhouse
		0·051	—	0·384	Greenhouse plants, not hardened
		0·071	—	0·682	Hardened in cold frame 2 weeks
Sweet potato	July	0·127	—	0·477	Garden plants
Kale	Oct.	0·223	—	0·511	
	Nov.	0·418	—	1·064	
Celery	Oct.	0·236	—	0·567	
	Nov.	0·423	—	0·793	

An examination of the data shows that the pentosan content of the succulents themselves is very variable, and that non-succulents may also contain large quantities. Among the latter a considerable proportion of the pentosans may be present in a water-soluble form. No data on the water-soluble pentosan content of succulents are, however, available. In succulent tissue the cell walls are thin and the protoplasm occupies a comparatively small proportion of the total cell cavity, the latter being mainly filled with sap. It would be expected that any extensive water-holding mechanism would be present in the sap itself and, if the pentosans play a part in the water relations of the cell, it is those soluble in water which are of the greatest interest in this connection. We do not know the nature of those pentosans hydrolysable by 1 per cent. HCl, or the part which they play in the cell, *e.g.* they might be present as food reserves, or even in combined form in the protoplasm, or as part of the wall structure. Nevertheless the pentosan theory of succulence has been based on the total pentosans extractable by 1 per cent. HCl.

In non-succulents the water-soluble pentosans may account for 0·5–5 per cent. of the dry weight. The 1 per cent. HCl-soluble pentosans may also be large in quantity, *e.g.* the leaves of the beech may contain as much as 9 per cent. of the dry weight. It appears unlikely that a slightly greater quantity of pentosan in succulents as compared to non-succulents is sufficient to explain their widely different features, especially when we consider the fact that some non-succulents contain more pentosan than some typical succulents.

Indeed McDougal and Spoehr (1918) state that it is only when the change from polysaccharides to pentosan is accompanied or followed by growth of cells, that succulence results. What causes this increase in size of cells is a question which remains unanswered.

Subsequently to the work of McDougal and his school, pentosans have been suggested as a factor causing hardiness in various plants, which enables them to tide over the winter period without injury. Thus Rosa (1921) concluded that the real difference between hardy and less hardy varieties is not merely a difference in the water content, but a difference in relative water-retaining capacity, and that this latter difference is due in large degree to the pentosan content. He conceives the pentosans, owing to their great power of imbibition, as holding, during freezing, sufficient water against the tendency towards ice formation to preserve intact the physico-chemical structure of the protoplasm. This theory has been very fully investigated by Doyle and Clinch (1926*a* and *b*) for conifer leaves, who found that the seasonal variation in pentosan content was quite opposed to Rosa's theory.

Further, Rosa claimed that the transpiration rate and the rates of drying were lower in his hardened plants than in the unhardened, implying that it was the capacity of the pentosans in holding water against forces of dehydration which caused this difference. Doyle and Clinch (1926*b*) investigated the rates of drying of various conifer leaves in an attempt to find a correlation between the rate of drying and the pentosan content. They failed to find any such correlations, and pointed out that the differences noted by Rosa might well be due to differences in leaf structure.

The fundamental assumption made in the pentosan theory of succulence is similar to that in Rosa's theory of hardiness in that both imply a capacity of pentosans to prevent loss of water. There is some experimental evidence bearing on this point. As early as 1904 Wisser found that no difference exists between the evaporation rates, under uniform conditions, of various plant saps and distilled water. Wisser found this to hold for plants from the principal ecological types. Spoehr (1919) determined the water-holding power of mucilage of specific gravity 1·017, placed in wide-mouth weighing bottles of approximately the same size. Comparisons were made with tap water. The bottles were placed on a table 1 metre in diameter revolving once every 2 minutes, and weighings were made every hour.

Loss per bottle per hour of mucilage solution and tap water.

Time (hrs.)	Mucilage	Water
1	2·4402 2·4157	2·4601 2·4187
2	3·1066 3·0895	3·1900 3·2112
3	4·6807 4·6653	5·2711 4·5691
4	5·2185 5·3573	5·5291 5·5844

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Some of the results are shown in the table. The difference is very slight during the first hour, after which evaporation is retarded through the formation of a slight film on the mucilage. It is very unlikely that such a film ever becomes operative in the case of plants, and even so it could only be formed at the transpiring surface.

There is thus no evidence that pentosans are able to hold water against forces of evaporation any better than plant sap free from mucilages, and the widespread idea that succulents maintain a slow rate of water loss and are capable of holding a large water reserve under conditions of extreme drought because of their pentosan or mucilage content is therefore without adequate foundation. As we have seen, the transpiration per unit area of many succulents is as great as, or even greater than, that of many mesophytes, which we should not expect to be the case if the mucilage supposed to be present had the capacity of holding water against forces of evaporation.

Bews and Vanderplank (1930) give data on the pentosan content of *Hypoxis rooperi* and *Portulacaria afra*, and also discuss some of the theoretical bearings of pentosan content on the water relations. They point out that, since the cell sap of succulents is in equilibrium, or always tending towards an equilibrium, with the protoplasmic colloids, the imbibition forces of the latter cannot be greater than the suction pressure of the vacuole. The osmotic pressure of succulents is low, and hence the imbibition forces of the protoplasmic colloids must be low. This is essential in a water-storage tissue, for it must not only be capable of storing water, but also of giving it up to less specialised tissues.

A point of considerable importance to the pentosan theory of succulence is the increase in pentosan content caused by a decreased water content of the cell, and by exposure to high temperatures. Bews and Vanderplank state that pentosans do not always increase with decreasing water content, for occasionally they have observed decreases in water content accompanied by decreases in pentosan content. It is also possible that small changes in pentosan content occur in non-succulent types, *e.g.* the stems of *Hypoxis* and *Portulacaria* (Bews and Vanderplank, 1930); the experiments of Doyle and Clinch (1926*a*) on conifer leaves and of Abbot (1923) on apple and peach also point this way. It may be therefore that succulents only show more marked changes in pentosan content because they are exposed to high temperatures and to large variations in water content. The presence of mucilages is to a large extent characteristic of other xerophytes (Cannon, 1924). The fact that the pentosan content of succulents varies so much in different plants, and in the same plant under different conditions (Spoehr, 1919), may itself be interpreted to favour the view that they are of no essential importance to a succulent structure. The most reasonable supposition is that the formation of pentosans is favoured by certain external conditions, and that in succulents there is more scope for these conditions to act. At least one of these factors, viz. exposure to high temperatures, is common to many other xerophytes, and may be a factor in the formation of mucilages in xerophytes in general.

As far as can be ascertained, there are no data on the pentosan content or pentose metabolism of halophytes.

Pentosans, once formed, may aid in the taking up of water by desert succulents during the rainy period, although they may play no important part in the subsequent retention of water during the dry period. It seems improbable, however, that the formation of pentosans is the direct cause of succulency, for a high mucilage content of the tissues does not of necessity indicate succulency. While some succulents, such as aloes, cacti, etc., have a highly mucilaginous sap, others, such as the *Sedums*, *Crassulas*, *Kleinia*, etc., have a very watery sap.

(4) *Causation of succulence in the wider sense.*

An explanation similar to that involved in the pentosan theory has been put forward as to the cause of succulency in the wider sense by Pearsall and Ewing (1929). Invariably associated with the increased juiciness and water content of radish, turnip, etc., caused by the application of nitrogenous fertilisers, they found a decreased hydrogen-ion concentration. They suggest that the difference in water content is caused by the difference in colloidal (protein) swelling, caused by difference in *p*H. Plants receiving abundant nitrogenous nutrients had relatively larger amounts of soluble nitrogen in the form of amines and amides than normal plants. Associated with the increased quantity of amino acids was a decrease in the gross sugar concentration. Assuming the sugars to be the mother substances from which the organic acids are formed, they suggest that the decreased acidity follows the decrease in the sugar concentration. The higher *p*H caused by the decreased acidity in turn affects the imbibitional properties of the proteins present, and according to these authors results in a higher water content, *i.e.* increased "succulency" of the tissues. In support of their view they state that when sets of high and low nitrogen plants were dried to constant weight and then exposed to moist air, the gain in weight due to absorption of moisture from the air was greater in the case of the high nitrogen plants.

Here again the water-holding capacity of colloidal protein solutions needs to be demonstrated. It is also probable that the water relations have been entirely changed by the application of large quantities of nitrogenous nutrients: indeed Pearsall and Ewing state that the rate of transpiration is much lower in the case of the high-nitrogen plants. It is therefore possible that the increased water content may be due to a shifting of the equilibrium between absorption of water and loss of water by transpiration. Again, the greater absorption of water by the dried tissues of the high-nitrogen plants when placed in moist air may well be due to the presence of greater quantities of salts of a hygroscopic nature, such as calcium nitrate. Unfortunately no data on the nitrate content of the high and low-nitrate plants are given; although the total water-soluble nitrogen is greater in the high-nitrate plants in accordance with this suggestion.

Chapman (1931) has recently described experiments with *Tradescantia fluminensis* in which succulence was produced by withholding iron, by insufficient nitrogen supply, and by excess of potassium salts, particularly if the calcium supply was diminished at the same time: if potassium was deficient as well as nitrogen, succulence did not develop. The succulent leaves produced under these conditions were three to four times as thick as the normal ones, the increase in thickness being mainly due to an increase in the size of a single layer of cells in the water-storage tissue, and the cuticle was nearly twice as thick as in the normal forms. He also concludes that pentosan content bears no relation to succulency, but is raised by nitrogen starvation. The development of succulence he attributes to the greater water-retaining power of compounds of the monovalent metals with the cell constituents, as compared with that of compounds of the divalent metals.

The complicated nature of the problem of succulency is evident from the results of Pearsall and Ewing on the one hand, who found that "succulence" was caused by the application of large quantities of nitrogen, and of Chapman on the other, who concluded that nitrogen deficiency was one of the factors causing "succulence."

All the theories which have so far been propounded seem to be inadequate to explain the succulent habit, and the observations of Harrison (1930), which showed that the succulent members of the Euphorbiaceae have a different basal number of chromosomes from the non-succulent members, will, if confirmed, only increase the complexity of the problem.

IV. THE ACID-METABOLISM OF SUCCULENTS.

(1) *Earlier work.*

A casual observation by Benjamin Heyne in 1819 first drew attention to the peculiar type of acid metabolism which has been found to occur in many succulent plants. Detailed researches by various investigators have established the fact that succulents are generally rich in organic acids, and that they show a diurnal periodicity in the quantity of these substances. Mayer (1875), Kraus (1886) and others have shown that there is a decrease in acid content during the day, followed by an accumulation of acid during the night. Kraus observed a decrease in acidity in several species of a non-succulent type during the day, and as a result made the mistake of assuming that the periodicity applies quite generally to non-succulents as well as succulents. Kraus (1886), and Purjewicz (1893), showed that the mineral salts of organic acids do not change materially from day to night, and thus proved conclusively that the daily periodicity is not due to the neutralising action of the bases derived from the soil. De Vries (1884) was the first to maintain that the formation and decomposition of acids is continually going on, and that the accumulation or loss of acid is dependent upon the relation between these two processes, *i.e.* at night the synthesis of acid is more rapid than its breaking down, and in the day the reverse is true. Kraus, Purjewicz and de Vries were of the opinion that carbohydrates were the mother substances from which the acids were formed. Purjewicz proved that leaves placed in sugar solutions showed increased formation of acids. Kraus found that the quantity of acid formed at night increases with increased intensity of assimilation during the day, and this result was confirmed by de Vries. The latter held, however, that the action of light is not due directly or solely to photosynthesis, for exposure to light in an atmosphere free from CO_2 causes a rise in acidity at night (Kraus had previously stated that this increase was very slight). De Vries therefore postulated a stimulation of the protoplasm by light, as a result of which acid was formed when the plant was placed in the dark. In accordance with this view he found that very weak light which could not cause marked photosynthetic action was able to promote nightly rise in acidity. A very short period of illumination, on the other hand, causes no rise in acidity at night.

Investigating further the effect of external factors on the acid formation in succulents, de Vries found that the decomposition of acid was mainly facilitated by light, but that prolonged darkness and exposure to high temperature were also effective. He held, however, that light was not the cause of deacidification, but merely promotes the process.

Light is not only concerned with the decomposition of the acids, but indirectly with their formation. Warburg (1886) was of the opinion that the nature of the rays of light was immaterial in the acid relations of succulents, whereas Mayer and Kraus maintained that only rays affecting assimilation of CO_2 were of importance in this respect.

De Vries showed that temperature was also an important factor in the formation and decomposition of acids. The minimum temperature at which acid formation occurred was 0° C., the maximum 38° C., and the optimum at which acid formation is most active, 13° C.

With regard to the relative importance of light and temperature in promoting deacidification, Richards (1915) showed that up to 20° C. light was the chief factor, temperature being of little importance up to this point. There was indeed little change in the rate of deacidification with rising temperature up to 30° C., but at 40° C. there was a very marked increase of rate. Hempel (1917) also established the greater importance of light within the ordinary range of temperature.

Aubert (1890, 1892*b*) considered that the process of acid decomposition could take place in two ways, determined either by the influence of light or by that of temperature. A rise of temperature should occasion a development of carbonic acid at the expense of the accumulated malic acid, and the influence of light give rise to a splitting up of the acid accompanied by the development of oxygen. This view was, however, challenged by Gerber (1896), who maintained that the decomposition of acid whether in light or in darkness was associated with the development of CO_2, and that the latter during exposure to light was directly used in the process of assimilation.

In addition to light and temperature long-continued darkness causes deacidification. Purjewicz showed that this decrease in acidity was not due to translocation or to neutralisation of the acid by bases derived from the soil. Astruc (1892) showed that etiolated tissue was much lower in acidity than that which came from normally illuminated plants. According to Purjewicz (1893), the period of darkness during which there is an increase in acidity varies from 8 hours in some species to 24 hours in others. He believed that these differences were correlated with differences in stability of malic, oxalic, tartaric and citric acids, since species do not all contain the same acids.

It has been shown that the formation and decomposition of the acids are also markedly affected by the oxygen supply. Warburg (1886) maintained that the decomposition of the acids is a purely oxidative process, since it is furthered or retarded by augmented or diminished oxygen supply. This view was supported by Purjewicz (1893), who showed that the breaking down of the acids is greatly inhibited in the absence of oxygen. He also found that a certain amount of oxygen is essential for acid formation, but that the quantity is much less than that required to break them down. Nevertheless he held that oxygen is of greater significance for acid formation than for deacidification. Astruc (1892), on the other hand, claimed that oxygen was so necessary for the production of acid that in atmospheres with less than the normal supply the process was greatly impeded: and that acidification is favoured if the oxygen supply is above normal. The work of Richards (1915) gave no support to Astruc's claims, for in material of low acidity he found no appreciable change when it was given an excess of oxygen, and in an atmosphere of hydrogen the accumulation of acid was essentially the same as in normal air. The action of oxygen was to facilitate the decomposition of acid as far as it could be carried. The effect of wounding (which facilitates access of oxygen to the tissues) was similar to that of oxygen in causing a decrease.

With regard to the relationship between the peculiar acid metabolism and succulency, Warburg (1886) suggested that acid formation and its periodicity were especially characteristic of plants which, by reason of their protection against high transpiration rate, are not favourably placed in relation to gaseous interchange. He pointed out that the breaking up of the acid affords an important saving of CO_2, since this takes place in the daytime when the liberated CO_2 will be utilised in the photosynthetic processes. Aubert (1890, 1892*b*) also concluded that the more succulent a plant is, the more acid it contains, and that succulency and acidity showed a direct correlation. Hempel (1917), however, while admitting that the power of producing and accumulating acid differs to a very high degree in different plants, found no correlation between the intensity of the acid metabolism and the degree of succulency.

Aubert found that in leaves and stems the acid content increased until these organs were mature, thereafter diminishing. Astruc (1892), however, concluded that the acids are formed most of all in young organs which show high cellular activity and a maximum turgescence, and that they tend gradually to diminish in amount as the tissues advance in age, either by combination with alkaline bases absorbed from the soil water or by esterification. Richards (1915) also found that, in the case of the cacti, the young joints not only show a higher acidity than the older joints from the same plant, but also show greater variations therein. He found that the cortex was more acid than the pith, while leaves were of the same acidity as the outer layers.

With regard to the nature of the acids present in succulents, special interest attaches to the early work on the Crassulaceae. Schmidt (Czapek, 1921) found that in *Bryophyllum* the calcium salts of malic acid, after exposure to light and darkness respectively, were not identical. Aubert (1890) thought that in cacti it is malic acid proper that is produced, but that in the Crassulaceae the substance is somewhat different and is to be regarded as isomalic acid. According to Hempel (1917) malic acid is the predominant acid in succulents, not only in the Cactaceae, but also in the Crassulaceae and *Mesembryanthemum* spp. The predominant acids in the metabolism of succulent plants are malic and oxalic, and to a much lesser extent, succinic. Bendrat (1929) has identified the acids present in many succulent plants, including "desert" succulents and epiphytic succulents, and her results are summarised in the following table.

		Direction of diurnal change
Sempervivum	Inactive-malic; *l*-malic; *d*-malic; succinic	+
Mesembryanthemum	Oxalic, malic	−
Bryophyllum	Malic	+
Epidendrum	Inactive-malic	+
Vanilla	Inactive-malic; *d*-malic; oxalic	+
Oncidium	Inactive-malic	−
Cypripedium	Oxalic; inactive-malic	−
Billbergia	,, ,,	+
Portea	,, ,,	+
Tillandsia	*l*-malic; inactive-malic	+
Cryptanthus	,, ,,	+
Nidularia	Oxalic; inactive-malic	−

+ =deacidification during day; − =acidification during day.

To these can be added the following:

Crassulaceae	Isomalic-acid (Mayer, 1878)
Opuntia versicolor	Oxalic; malic (Spoehr, 1913)
Rochea falcata	Malic; traces of citric and oxalic (Hempel, 1917)

(2) *Recent work.*

The acid metabolism of succulents and indeed of other plants has received considerable attention in recent years. This is partly due to an appreciation of the importance of hydrogen-ion concentration, and partly to the striking relationships that have been established between organic acids and certain vital processes in the muscle cells of animals.

Gustafson (1924, 1925) investigated the hydrogen-ion concentration of *Bryophyllum calycinum.* He found that on a cloudy day there was a *p*H gradient, the oldest leaves having the higher, and the youngest leaves the lower *p*H values. On a sunny day, however, the gradient was reversed, the oldest leaves now having the lower *p*H values. Gustafson established the normal periodicity, in sunny weather, both of the titratable acid and of *p*H, with a close correlation between the two. In cloudy weather the changes were smaller and the correlation much less close. (Small (1929) reproduces Gustafson's results in the form of curves.)

Gustafson (1925) found that, in continuous darkness for 15 days, the *p*H fell to a minimum of about 3·7 in the first 3 days, rose to about 4·5 on the sixth day and thereafter remained more or less constant. Lynn (Small, 1929) obtained similar results for the individual tissues of the leaves of the same plant, using the Range Indicator method. Ulehla (1928) gave some similar data for *Opuntia phaeacantha.* He obtained the following values of *p*H at different times of the day: 6 a.m.—3·5–3·8; 6.45 a.m.—1·4; 8.30 a.m.—4·5; 2.30 p.m.—6·0; 4.0 p.m.—5·5. He states that *Rheum undulatum* shows a similar diurnal periodicity in *p*H.

The metabolism of succulent plants has recently been investigated from a new point of view by Bendrat (1929). She criticises the previous methods of investigation as being inadequate to elucidate all the problems of the acid metabolism. Her investigations include determinations of the total acid (free and combined) present, but the actual method used in the determinations is not given. The determination of oxalic acid is of course an easy matter, but a quick and accurate method of estimating malic acid is not available. It seems that Ruhland and Wetzel's school have evolved such a method, which Bendrat used, but as far as can be ascertained, no details of it have yet been given in any of their publications.

As a very striking example of the incompleteness of estimations of acidity by titration, Bendrat shows that, in the case of *Sempervivum glaucum*, titration values indicated that the formation of acid was greatly hindered by lack of oxygen, but that oxygen had no effect on the total amount of acid present. The titration results are as follows (cc. molar alkali per 1 cc. expressed sap):

	Control	Vaselined leaves	Control	Vaselined leaves
Afternoon	0·024	0·024	0·032	0·030
Following morning	0·059	0·039	0·056	0·035

Vaselined leaves (without oxygen) thus failed to produce as much acid as the control leaves when titrimetric acidity was measured. The following are her results for the total acid:

	Control	Vaselined leaves	Control	Vaselined leaves
Afternoon	0·122	0·122	0·134	0·136
Following morning	0·131	0·131	0·145	0·145

Bendrat therefore claims that lack of oxygen has no effect on the accumulation of acid, and that it is only a component portion that appears to alter. It is this latter component that is measured by titration. She has also measured the diurnal variation in the total acid, and found that in general the morning value of the acid content was greater than that for the preceding evening, showing a gain in acid overnight. More acid was accumulated at 11° C. than at 22° C.; and under conditions of dull light the diurnal variations in acidity were not nearly so marked as on a sunny day. In the case of *Sempervivum glaucum*, old, middle-aged, and young leaves behaved differently in several respects. Old and middle-aged leaves always showed a higher acid content in the morning than in the evening, but differed as regards the actual increase overnight, *e.g.* in one case the morning value for old leaves was 10 per cent. higher than the evening value, while for middle-aged leaves, on the same date, the morning value was 67 per cent. higher than the evening value. The behaviour towards temperature also differed, mainly as regards the quantity of acid formed at different temperatures. The direction of change is, however, similar for old and middle-aged leaves, and they behave like other succulents, being normal in the direction of their diurnal periodicity.

The young leaves, on the other hand, do not show the normal periodicity—sometimes showing an increase during the night, but at other times a decrease. The diurnal change of total acid, and the effect of temperature on acidity, are followed by similar changes in the titratable acid. In *Sempervivum glaucum* the acid was mainly malic, with a small quantity of succinic, and oxalic acid only in traces.

Bendrat also determined separately the malic acid present as the optically active and inactive forms. In old leaves about one-third of the total acid was present in the optically active laevo form, the rest being optically inactive. There was optically active acid present both morning and evening, but changes in the optically active part were not correlated with changes in the total acid content. In middle-aged leaves the dextro-rotatory form was present in addition to the laevo and inactive forms, while in young leaves the only optically active form was the laevo form.

The nature of the acid present in a number of other plants, both succulents and non-succulents as determined by Bendrat, together with the direction of their diurnal change, are given in the table on p. 196.

From Bendrat's work it appears that *Mesembryanthemum* has a negative diurnal change, *i.e.* the acidity is greater in the evening than in the morning. This is true also of young leaves of *Sempervivum glaucum*. The epiphytic succulents showed a positive diurnal variation, while the non-succulents examined in general showed an active acid metabolism in an opposite direction to that which is typical among succulents.

Bendrat concluded also that if the evening value of acidity was low, there was generally a large increase during the night, in the case of succulents. In the course of her work she came to the conclusion that the acid metabolism of succulents is affected by many internal factors, which may be of an importance equal to, or greater than, that of the external factors. Some of these internal factors are probably of a nutritional character, while the degree of development of the organ is also of importance.

The data available on the acid metabolism of the succulent halophytes are extremely meagre. In fact, apart from that of Clarke on the marine algae, there is little information on the acidity of maritime plants in general. Delf (1912) states that the observations of Wolff and others show that, in halophytes, malic acid is present in small amounts in addition to chlorides. Small (1929, p. 122) states that *Salsola kali* is well known as an "alkaline" plant, meaning by this that the *p*H of the tissues is above 5·5. Small infers that there may be succulence without accompanying acidity of the tissues.

It appears then that the halophytic succulents do not show the active acid metabolism characteristic of other succulents, though further investigation is necessary before this generalisation can be established. The epiphytic succulents according to Bendrat are similar to the xerophytic succulents in showing an active acid metabolism with its characteristic periodicity. All the "xerophytic" succulents, however, do not conform to the diurnal acid changes which are characteristic of the majority. The young leaves of *Sempervivum glaucum* and *Mesembryanthemum* as a whole, according to Bendrat, do not show the diurnal variations. Hempel (1917), too, found that in *Mesembryanthemum echinatum* and *M. linguaeforme*, the buffering at the natural *p*H was less in the dark than in the light, probably implying a decrease in total acid content in the dark, since the acids are responsible for the main buffer action in this region.

(3) *Acid metabolism in non-succulents.*

The majority of non-succulents do not show an active acid metabolism such as occurs in the non-halophytic succulents. It is generally the case that the acid metabolism is very feeble, and there is no periodicity as occurs in the succulents. Usually the acidity is slightly higher when the plants are exposed to light, decreasing in the dark. There are some non-succulents which show an active acid metabolism however. Steinman (1917) has worked on such a type, viz. *Rheum* sp. He found that the influence of light was to increase the acidity in all cases, and darkness invariably caused a decrease. He showed that the acid metabolism was entirely different to that in succulents. Ruhland and Wetzel (1927) have also investigated the acid metabolism of *Rheum hybridum*. They found very little acid in the lamina, with no well-defined changes, a result somewhat surprising in view of Steinman's results. Ullrich (1926) examined the changes in the total quantity of organic acids in the leaves of *Lactuca sativa* and other plants during the day and night. Bendrat (1929) states that the non-succulents examined by her (*Oncidium*, *Cypripedium*) have a distinct acid metabolism, but in a direction opposite to that of succulents. Her qualitative data for *Tillandsia*, *Cryptanthus*, indicate, however, a possibility of their possessing a similar type of metabolism to that of succulents. Clarke (1917), in his work on marine algae, showed that *Iridaea laminarioides* and *Gigantina exasperata*, which inhabit the shore, display diurnal variations of acidity similar to those of succulent plants, the acidity being highest in the morning, decreasing towards the end of the day, but sometimes rising before night.

(4) *Theories of formation of organic acids in plants.*

The first view of the origin of plant acids was that put forward by Liebig in 1840, which suggested that the acids were intermediate products in the synthesis of sugars from CO_2 and water. The main lines of evidence for his theory was the decreased acidity accompanied by increased sugar content in ripening fruits, and also in germinating seeds, and the decreased acidity of succulents in sunlight, also accompanied by increase in sugar content. Warburg (1886) pointed out that the acid diminution and sugar increase are not parallel phenomena, which makes it unlikely that the acids are converted to sugars. Warburg also held that the diminution in acids with ripening follows the development of the organ, accompanied by a fall in the intensity of respiration, so that, after the active decomposition of the acid into CO_2 and water, acid formation does not keep pace with the accumulation of sugar (by translocation and dissolution of starch).

The work of Mayer (1875, etc.) and de Vries (1884) was the first really serious objection to the Liebig theory. They pointed out that previous exposure to light was necessary before acid formation occurred. De Vries also claimed that the deacidification in light was in itself an important fact against the view that the acids are intermediate products in the formation of sugars.

Warburg (1886) interpreted his results on the effect of oxygen on the process of deacidification, as supporting the oxidative nature of that process. Finally

Purjewicz (1893) proved by nutrition experiments that the formation of the acids is dependent upon the carbohydrates present.

Berthelot and André (1886) supported the Liebig view, pointing out that the foliage leaf is a typical reduction organ. They interpreted their results on *Rumex acetosa* as indicating the formation of oxalic acid by reduction of carbonic acid in the leaf. Brunner and Chuard (1886) also regarded the plant acids as products of a reduction process taking place in assimilating organs under the influence of light. More recently Steinman (1917) concluded from his investigations on *Rheum* that in plants with a very acid sap, other than succulents, the organic acids are formed along with carbohydrates as assimilatory products.

The other early view of importance was that put forward by Mayer (1875), and was in essence that the organic acids arise in the respiratory processes as incomplete products of oxidation. This view was later supported by Kraus (1886) who further developed the hypothesis, and who held that the decomposition of the acids in light had no immediate connection with the true respiratory activity; and by de Vries with the proviso that a previous light stimulus of the protoplasm was necessary for their formation. Warburg's results on the effect of oxygen also led him to support the hypothesis. Warburg, and also Aubert (1892*a*), regarded the acid formation and its periodicity as being characteristic of those plants which by reason of their protection against high transpiration rate are handicapped as regards gas interchange. Aubert regarded the succulence itself as inhibiting the free passage of gases between the tissues and the external atmosphere. These investigators regarded the incomplete oxidation as being due to the poor oxygen supply caused by the anatomical features of succulents. Richards (1915) also accepted this view, but he added that since acid formation occurred even in complete absence of oxygen, they may also be formed in the course of anaerobic respiration. Richards also regards the deacidification in sunlight as being entirely outside the true process of respiration.

Steinman (1917) has adversely criticised the view on the ground that with rising temperature the acids decrease in amount, whereas the rate of respiration increases. If the acids were formed in the respiratory process, Steinman holds that the quantity of acids should increase with rising temperature. He therefore concludes that their formation is not related to respiratory activity.

A third view of the origin of plant acids was put forward by Palladin in 1887, though Holzner had stated a similar view twenty years previously. This view was that organic acids appear in plant organs as by-products in the regeneration of proteins from asparagine and carbohydrates.

The more recent work on the acid metabolism of succulent plants has brought out many interesting relationships. Especially noteworthy is the work of Bennet-Clarke (1930), in that it makes it at least possible that changes postulated both in the Liebig hypothesis and in the Mayer-Kraus theory can take place in succulent plants. Using two species of the Crassulaceae, he found that large quantities of acid formed at 2° C. rapidly disappeared when the temperature was raised to 27° C. This disappearance could not be wholly accounted for by conversion of acid into

CO_2, as the carbon changed from the form of malic acid was three times that evolved as CO_2. It was found that the malic acid was converted into a carbohydrate, and further that the carbohydrate formed was a heptose sugar. Bennet-Clarke concludes from these and other results that the carbon passes through a series of cyclical changes from carbohydrates to products of glycolysis, and then to malic acid, which is partly resynthesised to carbohydrates, a process similar to that in muscles whereby carbohydrate is converted to lactic acid, part of which is further oxidised to CO_2, while part is converted back to carbohydrate.

Kostychev (1927) has very adversely criticised the view that plant acids arise by incomplete oxidation in the respiratory processes, maintaining that Mayer's view depends on observations too weak to support it. He holds the view that the plant acids represent either transformation products of amino acids, or either normal or by-products of incomplete change of sugar to amino acids in the course of protein synthesis. He suggests that the greater part of the malic acid of seed plants is formed in the process of formation or deaminisation of asparagine. Under conditions of nitrogen deficiency, he holds that all the malic acid cannot be worked up into asparagine, and the excess is then destroyed by oxidation processes leading to the formation of CO_2; but he considers that this is not a direct or normal respiratory process.

A similar theory to that of Kostychev has been propounded by Ruhland and Wetzel (1926) and further developed by Wetzel (1927). The basis of their view is that the organic acids arise by deaminisation of proteins and amino acids. The acids are the nitrogen-free residues, or arise from nitrogen-free residues, of the deaminisation processes. That there is a certain connection between deaminisation and the formation of acids, they argue, is shown when *Aspergillus niger* and *Penicillium glaucum* are grown on peptone cultures, when there is evident a direct connection between ammonia formation (which is a measure of deaminisation) and oxalic acid formation. The deaminisation, they consider, may be a hydrolytic process, or an oxidative process, or both. In both hydrolytic and oxidative action the products will be nitrogen-free residues, and ammonia. Ruhland and Wetzel (1926) have demonstrated a correlation between organic acid and ammonia formation in *Begonia semperflorens*. They have further (1927) divided plants into two classes, (*a*) weakly acid plants, which they claim are also rich in amides, and (*b*) strongly acid plants, which are rich in ammonia. Rhubarb, which is a typical example of the latter class, was investigated by these authors, the results being interpreted as being in entire agreement with a formation of organic acids by deaminisation of proteins and amino acids. They point out that the formation of acids, together with ammonia, render the latter non-poisonous, and is to be regarded as an automatic control. They suggest that the Mayer-Kraus theory was generally accepted only because no other process except respiration was regarded as of sufficient intensity to render comprehensible the quantities of acid which appeared, but that their researches on *Rheum*, and also on *Begonia* show an intensity of nitrogen metabolism of comparable magnitude. Their researches point to malic and succinic as the acids first formed, the oxalic acid arising later from these acids.

It is impossible to discuss the theory fully in all its aspects at the present stage, owing to the limitations of the data available, but it does seem likely that there is a connection between nitrogen and acid metabolism in these strongly acid plants. Whether such a connection holds in succulents remains for further investigation.

Although the acid metabolism of succulents has received so much attention, the incompleteness of the data is still obvious from the fact that such widely different views of the origin of plant acids are still held. Mayer (1926) supports the view that the formation of malic acid is a part of the up-grade metabolism, being indeed an evanescent intermediate product in the ordinary assimilation process.

(5) *Chemical evidence bearing on the acid metabolism of succulents.*

The first relevant evidence was that of Purjewicz (1893), who found an increase in the quantity of volatile acids, such as acetic and possibly formic acid, during deacidification. That the decomposition of the acids actually does take place by formation of volatile acids was again shown by Spoehr (1913), who found that the photolytic action of light on malic acid results in the formation of a number of degeneration products including formaldehyde, acetaldehyde, formic acid, acetic acid, glycollic acid, oxalic acid, and carbon dioxide. The malic acid breaks down step by step to form simpler derivatives, accompanied by a constant evolution of CO_2. That the disruption of the acid should be rapid at first, becoming gradually slower, is to be expected from the greater stability of the simpler acids, especially formic acid. This photolytic action was demonstrated *in vitro* with dilute solutions of malic acid. Spoehr, however, carried out some interesting experiments with the plant juices. He failed to show that the deacidification was caused by an enzyme; indeed, he found that it is not wholly dependent on the living protoplasm, since the expressed juice when placed in sunlight diminished in acidity with the formation of CO_2. The process of deacidification was found to be greatly intensified by adding a small quantity of those substances in the cell sap (of *Cactus*), which are precipitated by alcohol. These substances are active when employed in the raw, boiled or calcined state, and Spoehr concludes that it is the influence of salts from the plant juice which here makes itself apparent, and that there can be no question of any enzymatic effect.

In connection with the formation of malic acid Baur (1913) states that glycollic acid can condense to form carbohydrates when exposed to light with metallic salts, the photolysis giving formic acid and formaldehyde, and substances like pentoses and hexoses. Baur states that malic and citric acids are also condensed from glycollic acid, and he suggests that malic acid arises in this way in succulents. Bloor (1912) claims that the tissues of maple are capable of building up sugars from malic acid by means of an enzyme. The interpretation of his results, however, affords some difficulty.

V. THE RESPIRATION AND GAS EXCHANGE OF SUCCULENTS.

The possibility of an abnormal type of gas interchange in succulents was recognised even before the peculiar acid metabolism had been established. It was based, however, upon an isolated fact discovered by de Saussure (1804) that in an *Opuntia* with which he experimented the respiratory coefficient fell far below unity, *i.e.* under some circumstances the intake of oxygen greatly exceeded the evolution of carbon dioxide. The significance of this fact was not understood at the time, and it was left to later workers to relate it to the peculiar type of acid metabolism. Mayer (1878) showed that deacidification in light was accompanied by evolution of oxygen, and suggested that the oxygen was formed in the photosynthesis of carbon dioxide produced by the decomposition of the acids. Purjewicz (1893) found that the CO_2/O_2 ratio was lowest at the periods of maximum acid formation, and highest when the acid is being broken down. Similarly deacidification in continuous darkness is accompanied by an increase in the CO_2/O_2 ratio. Astruc (1892), indeed, stated that any cause which tends to hasten deacidification tends also to increase the CO_2 output and to raise the ratio.

Aubert (1892*b*) held that the more succulent a plant is, the more acid it contains, and it will absorb in the dark an increasing amount of oxygen with a minimum output of CO_2, as a consequence of which its gas-interchange ratio falls. He argued that succulents produce malic acid instead of liberating CO_2, and when with higher temperature or other causes the formation of malic acid is inhibited, more of this gas is produced in proportion to the oxygen absorbed. He regarded the abnormal acid metabolism and gas interchange as being directly caused by the anatomical and morphological features of succulents, which in turn resulted from their special type of environment.

Working with cacti, Richards (1915) found a close correlation between the respiratory coefficient and the acidity of the tissues. With rising acidity there is a rising ratio, until when the acidity is highest the ratio is slightly more than unity. From a closer examination of the data, however, he concluded that the variation in CO_2/O_2 ratio at any given acidity is very largely dependent on whether the acidity happens to be rising or falling.

With regard to the relationship between deacidification and photosynthesis, Aubert (1892*b*) found that succulents are able to give off oxygen in light of sufficient intensity, even in an atmosphere devoid of carbon dioxide. In light, evolution of both CO_2 and oxygen frequently occurred. The evolution of oxygen in an atmosphere devoid of carbon dioxide was affected by both light and temperature. Thus oxygen was given off at low temperatures in diffused light, at ordinary temperatures in bright diffused light, and at high temperatures in brilliant sunlight. These results have been largely confirmed by Richards, who suggested that the evolution of CO_2 in light represents the excess of that formed in the deacidification process over that utilised in photosynthesis. Richards also found a high rate of CO_2 evolution in an atmosphere of hydrogen and of nitrogen. The acidity did not diminish at the same rate under these conditions as it does when oxygen is available, and he suggests that the CO_2 evolved does not arise from the decomposition of acid to the same extent as under normal conditions.

Maquenne and Demoussy (1913) explain the abnormal gas interchange of succulents on more purely physical grounds. The lower value of the respiratory quotient at night, when the acidity is rising, they ascribe largely to the increased solubility of carbon dioxide in the juices of the plant at the lower temperatures which prevail at night. This would cause an apparent fall in the ratio by diminishing the amount of carbon dioxide evolved from the plant. Richards (1918*b*) also obtained evidence that considerable quantities of CO_2 are dissolved in, or in some way occluded by, the fleshy tissue in *Mesembryanthemum* and *Dudleya*. It is very doubtful, however, whether this increased solubility could possibly account for enough of the CO_2 to produce the considerable effect on the gas ratio which has been demonstrated.

Spoehr (1919), investigating the anaerobic respiration of cacti, found that the CO_2 emission under anaerobic conditions was the result of carbohydrate respiration. Under aerobic conditions there was no accumulation of alcohol during the night coincident with the nocturnal acidification, and he therefore concluded that normal respiration was not intramolecular in nature. Under anaerobic conditions, on the other hand, there was a very active production of alcohol.

The relationship between the respiratory quotient and the acid metabolism in succulents has afforded most of the support for the view that organic acids arise as intermediate or incompletely oxidised products of respiration. It is for this reason that in the earlier accounts of the metabolism of succulents, and indeed in some of the later ones also, the acid metabolism and respiration are discussed together as being merely two intimately related aspects of the same phenomenon. This phenomenon was almost universally believed to be caused by the morphological and anatomical features of succulents. The view is still held by many modern investigators, *e.g.* Spoehr (1919, p. 66), who states: "The characteristic formation of acids is intimately associated with the restricted oxygen supply consequent on the structure of the succulent type." The only controversial point was the connection between deacidification and the true respiratory processes. Nathansohn (1910) regards the deacidification as simply a second step in the katabolic changes, and as part of the true respiratory process. Richards, however, does not regard deacidification, and the evolution of carbon dioxide accompanying this process, as being connected with any of the actual vital processes; the breaking down of the acid in sunlight being an unavoidable consequence of its accumulation in the tissues.

Discussion of the view that the peculiar metabolism of succulents is caused by difficulty of gaseous interchange inherent in the succulent structure. The chief of the structural modifications which have been held to hinder gaseous exchange are (*a*) modifications of the external surface, (*b*) the character of the internal tissues.

Since the rate of transpiration of many succulents per unit area of surface may be relatively high, it would seem that the net result of all the structural modifications of the surface in impeding the escape of water vapour from these plants is not large. It seems likely therefore that the gas interchange per unit area of surface is not greatly affected by these structural features. The decreased ratio of surface to bulk might, however, cause the actual amount of the gas interchange to be small in comparison with the mass of the tissue.

The internal tissue of succulents is described as being composed of large cells filled with a watery sap, and when turgid pressing closely against each other, associated with which is a poorly developed air-space system. It is doubtful, however, whether succulents have a poorly developed air-space system. Aubert states that in certain cacti, and other fleshy Cactaceae, the internal atmosphere may compose a quarter, a third or sometimes more, of their total volume. Nevertheless, such a tissue has been held to offer a serious obstacle to the passage of gases and to cause a deficiency of oxygen in the tissues, as a consequence of which incomplete oxidation takes place, resulting in the formation of organic acids.

In order to obtain some idea of the effectiveness of these features in preventing gas interchange, some knowledge of the composition of the air in the tissues, especially during the period of acid formation, is essential. Full data of this kind are not available, though Aubert (1892*b*) gives the results of a large number of analyses. His results for *Crassula arborescens* show a high CO_2 content in strong light, probably due to decomposition of the acids. Under cloudy conditions the CO_2 content is much lower. The most remarkable point to consider, however, is the fact that the oxygen content is generally high, even higher than that of atmospheric air in many cases, and that the oxygen content increases during the day as a result of photosynthesis. We are thus led to the conclusion that acid formation begins in the evening when the oxygen content is high. It is difficult to see therefore how Aubert's own data support his contention that acids are formed because of a lack of oxygen in the tissues.

Of interest in this connection also are the results of Rivière and Pichard (1926) who found the composition of the gas drawn from apples to be 32·2 per cent. CO_2, 10·3 per cent. O_2 and 57·5 per cent. N_2.

Magness (1920) found that the composition of the gas in the intercellular spaces of apples, potatoes and carrots varied greatly with the temperature. The percentage of CO_2 increased from 5 per cent. at 2° C. to 30 per cent. at 20° C. in the case of the apple, and the oxygen similarly dropped from 14·2 per cent. at 2° C. to 5·0 per cent. at 20° C. In such organs no photosynthesis occurs, and the percentage of CO_2 is much higher and that of oxygen much lower than Aubert found for succulents. If lack of oxygen is the cause of acid formation, we should expect such organs with their large store of carbohydrate, and such a low concentration of oxygen in the intercellular spaces to show very active acid metabolism, but such is not the case, the acidity of the apple decreasing markedly during ripening.

Recent work on the absorption of gases by fluid surfaces is tending to modify greatly the conceptions generally held on gas-interchange relationships between phases. Schroeder (1924) and Romell (1928) have emphasised the very considerable resistance to the passage of carbon dioxide from the internal atmosphere of the assimilating leaf into the moist surfaces of the cells, as compared to the resistance to gaseous diffusion in the intercellular spaces themselves. Calculating from dimensions given by Schroeder for the ivy leaf, Romell estimates that the concentration of CO_2 in the air-space system of the leaf would be nowhere more than 1 per cent. below that in the external atmosphere. The data were calculated on the assumption that the hydrophase consists of pure water, which is probably not the case. Romell has therefore also calculated the concentration of CO_2 at different points in the tissues, assuming the solubility to be twelve times greater than that in pure water, with the following results:

	CO_2 concentration % of that of air (external atmos. = 100 %)		CO_2 concentration (difference)	
	In air space	In moist cell surface	% drop in air spaces	Air / Hydrophase
At under end of palisade	97·86	45·78	2·14	52·08
At the middle of the palisade	93·99	43·97	3·87	50·02
Just underneath upper epidermis	92·72	43·37	1·27	49·35

Even after making this large allowance for greater solubility of carbon dioxide in the cell-wall fluids, the fall in CO_2 concentration in the air-space system is small. Brown and Escombe (1900) drew a similar conclusion from a comparison between the rates of photosynthesis actually observed, and the rates of diffusion which stomata of given dimensions and frequency should make possible. It is obvious that similar factors must control the passage of oxygen, although allowances must be made for differences of concentration in the external atmosphere, smaller solubility, different coefficient of diffusion, etc. We have no evidence that oxygen is more soluble in the tissues than in pure water. It seems likely therefore that the resistance to entry into the internal hydrophase will be proportionately larger, and that the drop in oxygen concentration in the intercellular spaces will be smaller.

In so far as these considerations are valid, the resistance to entrance of gases into the internal hydrophase may be as high as or higher than the resistance due to the morphological and anatomical structure of succulents. The former resistance is common to all tissues whether of a succulent or non-succulent type, and it is possible that the influence of the structure of succulents on the availability of oxygen to the tissues has been greatly exaggerated. Even if these data were not available, the evidence for the view that the peculiar metabolism of succulents is due to incomplete oxidation of sugars is not at all strong. One of the main facts brought to support this view was the effect of oxygen on acid formation, but even this claim is now open to doubt in view of Bendrat's result (1929) that lack of oxygen does not affect acid formation when the total acid is measured, only the titratable component being affected by oxygen concentration.

The direct correlation of succulency with the degree of the abnormal metabolism, postulated by Aubert, has also been disproved by Hempel in so far as the intensity of the acid metabolism is concerned. Again, Richards (1915) showed that the cacti evolve carbon dioxide very actively, and if gases can pass quickly from the internal tissues to the external air, we should expect the converse process to be equally possible. Thus there is really little support for, and considerable evidence against, the view that the abnormal metabolism of succulents is due to incomplete oxidation, consequent upon a deficiency of oxygen in the tissues, which in turn is a result of the succulent structure.

Even so, however, this does not of necessity mean that there is no relation between the gas interchange and the acid metabolism. Indeed the close correlation which has been established between variations in acidity and change in the respiratory coefficient suggests that the two are intimately connected. It seems that we have not as yet got to the root of the connection between acid metabolism and the respiratory coefficient, nor to the mechanism by which the acids are formed. It is of interest in this connection that Ruhland's school have commenced a re-investigation of the respiration of acid-producing plants, but so far only a paper on their method (Ullrich and Ruhland, 1928) together with a few results have been published.

In summarising, we may state that succulent plants show a peculiar type of gaseous exchange, characterised especially in the night by a respiratory quotient which is less than unity. The respiratory ratio itself shows variations which are related to the periodicity in acid formation. It is thus probable that the gaseous exchange and the acid metabolism are intimately related. That both the variation in the respiratory quotient and the acid periodicity are two effects of the same cause appears to be the case, but that this cause is incomplete respiration due to deficiency of oxygen in the tissues does not seem likely.

VI. HYDROGEN-ION CONCENTRATION AND BUFFER-SYSTEMS IN THE CELL-SAP OF SUCCULENTS.

It is only within the last twenty years that the great importance of hydrogen-ion concentration in the metabolism of plants has been adequately appreciated. Since many succulents show large and sudden changes in their acid content, the question of the control of hydrogen-ion concentration, or buffer action, in their cell sap is of especial interest. It was Hempel (1917) who first investigated the buffer systems in the sap of succulents. The *p*H of sap extracted from leaves was found to lie in the range 3·9–5·7, even when the leaves had been exposed to light and darkness. This shows that although large changes in acid content occur, the hydrogen-ion concentration is maintained within a relatively narrow range. Hempel found that the main buffer system at the natural *p*H region consisted of the salts of malic or other organic acids produced in metabolism.

Theoretical considerations lead to the conclusion that malic acid exists free in the presence of its salts only at a *p*H below 3·95. As the *p*H of the succulents examined by Hempel was never below *p*H 3·9, it follows that it is a mixture of acid and normal malates which is responsible for the buffer action in succulents. This system is only effective up to *p*H 6·0–6·5, since it consists wholly of normal malate above this point. The actual strength or capacity of the buffer system is of course dependent upon the concentration of its constituents, and it is the latter which show a periodicity in succulents. It may thus be looked upon as an automatic control, since the more malic acid is produced the more efficient does the buffer system become, and any injurious effect which may be caused by sudden changes in acidity is thus minimised. Hempel attributes the considerable buffering above *p*H 6·0 which enters into all titration work with the sap of succulents to be in the main due to precipitation of aluminium malate, but partly also to certain unknown complex substances which were precipitated. She describes the latter as substances of a highly inconstant nature which act as acids above *p*H 4·7.

The work of Gustafson (1925) on the sap of *Bryophyllum calycinum* shows that, in this succulent also, malates are probably the main buffers at the natural *p*H of the sap. Armstrong (1929*b*) showed that in some of the acid non-succulents the buffer system consisted in the main of the salts of organic acids, together with some phosphate.

The p*H of the cell sap of succulents*. The *p*H of the expressed sap as measured by Hempel and others is only the resultant of the sap of different cells which might be originally different. So also the buffer systems which have been analysed represent a mixture of the contents of different cells. It does not follow that the results obtained give a true indication of the properties of the cell sap of intact cells.

With regard to the *p*H, Small (1929) has introduced a method to overcome this uncertainty, and to determine the actual *p*H of the cell sap in the intact cells. The striking feature of Small's results is that most succulents are remarkably uniform in their reaction, being usually within the range 4·8–5·2. There are, however, some exceptions, *e.g. Aloë variegata*, *Gasteria verrucosa* and *Salsola kali* have an "alkaline" reaction (*i.e. p*H above 5·5). Among those which have a lower *p*H than is usual among succulents are *Puya* leaf (*p*H 4·0 in mesophyll), *Mesembryanthemum stelligerum* and *Crassula rosea*. On the whole, however, the occurrence of a *p*H of 4·8–5·2 is characteristic of the non-halophytic succulents.

VIII. CONCLUDING REMARKS.

Having thus examined the data on the more outstanding features of the succulents as a class, it is obvious that we cannot point to any single feature as defining succulency. There are numerous features both of structure and of metabolism which are characteristic of a large number, but there is not a single feature yet investigated which can be stated to be general to all succulents. Likewise none of the theories which have been put forward to explain succulency are of general application. The class itself is characterised by a collection of features, morphological, anatomical and physiological.

Even the general make-up of the tissues is itself in essence a metabolic problem, and we have as yet no definite evidence as to what metabolic characteristics are expressed in this type of make-up, although the pentosan theory is an attempt in this direction. Water storage seems to be the main function, but extensive storage of mineral salts also occurs.

Other characters of importance, but not wholly general among succulents, are the periodicity in acid metabolism, and the abnormal gas-interchange relations.

That these are not usually regarded as being of equal importance to the structural features may be surmised from the fact that the halophytic succulents are classed with the other succulents, although the special type of acid metabolism and gas-interchange relationships have not been established for them.

Succulency must mean something definite and characteristic in the type of metabolism, which, unlike many other features, is not defined by family relationships, but which must nevertheless be capable of hereditary transmission. It remains for further research to discover what this is.

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FACTEURS BIOLOGIQUES ET PSYCHIQUES DE L'IMMUNITÉ

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I. INTRODUCTION.

LE problème de l'immunité est l'un des plus grands et des plus importants problèmes de la biologie moderne.

La découverte des sérums curatifs et des différents vaccins contre les maladies les plus dangereuses est le résultat de l'étude et de l'application des remèdes naturels et des moyens de défense que l'on trouve dans l'organisme même et qui sont susceptibles de lutter avantageusement contre le virus.

On distingue ordinairement deux sortes d'immunité: l'immunité naturelle et l'immunité acquise.

L'immunité naturelle est un état de résistance que certains individus possèdent contre quelques microbes ou certaines maladies. Par exemple, l'homme est réfractaire à la peste bovine, au choléra des poules; beaucoup d'animaux sont réfractaires à la syphilis, au paludisme, etc. L'exemple le plus démonstratif de l'immunité naturelle absolue nous est donné par les chenilles de la mite des abeilles (*Galleria mellonella* Linné). Ces chenilles résistent très bien aux maladies les plus terribles: tuberculose, diphtérie, tétanos, etc. Elles ne s'infectent pas même dans le cas où on leur injecte dans la cavité du corps des quantités formidables des microbes les plus pathogènes. Le bacille tuberculeux, en émulsion très épaisse, injecté dans la cavité du corps, est phagocyté et digéré en 2–5 jours (Metalnikov, 1927).

L'immunité acquise est un état de résistance que l'individu acquiert par le fait d'avoir subi une infection et de l'avoir surmontée.

L'immunité acquise peut être provoquée artificiellement par l'immunisation ou par la vaccination. On peut facilement immuniser un cobaye contre la diphtérie ou le choléra en lui injectant des microbes de ces maladies, atténués par un chauffage à 60°, ou par d'autres procédés. Dans une série de travaux nous avons démontré que les insectes s'immunisent avec une rapidité extraordinaire (en 10–24 heures) contre différents microbes, pathogènes pour eux.

Mais quelle est la cause de l'immunité naturelle ou acquise? Quels sont les facteurs de l'immunité?

Toutes les théories de l'immunité, émises jusqu'à présent, s'efforcent d'expliquer ce phénomène par l'existence de quelques facteurs autonomes agissant à l'intérieur de l'organisme.

Les uns, comme R. Koch, Baumgarten, Ziegler, Weihart, Behring, Pfeffer, etc., voient la cause de l'immunité dans l'action bactéricide des humeurs et du sang de l'organisme (théories humorales).

D'autres considèrent avec Metchnikoff et ses élèves que le facteur principal de l'immunité est le phagocyte, cellule vivante, qui englobe les microbes et les digère.

Tandis que les uns attribuent la plus grande importance, dans l'immunité, à la phagocytose, les autres considèrent que le facteur principal de celle-ci, particulièrement de l'immunité acquise, est l'anticorps.

Malheureusement toutes les théories actuelles sur l'immunité ont été créées principalement par l'étude des phénomènes de l'immunité chez les animaux supérieurs possédant du sang et un système d'organes compliqué. Cependant l'immunité, comme dans son temps l'a justement signalé Metchnikoff, est un caractère propre à tous les animaux, supérieurs et inférieurs, et même aux plantes.

Pour comprendre l'immunité, facteur biologique général, il faut créer une théorie qui nous explique non seulement l'immunité des animaux supérieurs, mais aussi l'immunité, caractère commun à tous les organismes vivants. Dans une série de travaux nous avons étudié l'immunité chez les organismes unicellulaires, les embryons des oursins, quelques vers et surtout chez les insectes. Nous avons cherché à démontrer les caractères communs à l'immunité chez tous ces animaux (Metalnikov, 1926).

Nous essayons d'étudier l'immunité en tant que réaction de défense de l'organisme. Les réactions de défense ne sont, au fond, que le résultat d'une propriété commune à tous les êtres vivants, qu'on appelle l'instinct de conservation.

L'instinct de conservation pousse l'organisme vivant à lutter pour son existence, à éviter les conditions nuisibles, à trouver et à choisir les conditions les plus favorables, et à défendre son corps contre la quantité innombrable des parasites internes et externes.

On ne peut pas s'imaginer la vie sans cet instinct et sans les réactions de défense. *C'est le caractère essentiel et principal des organismes vivants.*

Les organismes les plus simples, tels que les microbes, possèdent leur immunité. Pour se défendre contre les phagocytes, les microbes, ayant pénétré dans l'organisme animal, sécrètent souvent diverses substances toxiques qui détruisent ou repoussent les phagocytes. Certains microbes se forment des enveloppes ou capsules glaireuses qui empêchent leur englobement par les phagocytes.

D'un autre côté, l'organisme lui-même se défend contre les microbes en produisant toute une série de réactions de défense qui est à la base de l'immunité naturelle et acquise.

Les réactions défensives peuvent se produire soit à l'extérieur, soit à l'intérieur de l'organisme.

Les réactions externes ont lieu quand les microbes ou diverses substances étrangères rencontrent les muqueuses du nez, des yeux, de la gorge, etc.

Les cellules réagissent alors, soit par une sécrétion glaireuse, des larmes, etc., soit par l'éternuement, la toux, qui favorisent l'expulsion de ces substances.

Les réactions défensives internes sont beaucoup plus compliquées. Elles ont pour cause le passage des microbes dans le sang, dans les organes et les cavités internes.

Tout organisme est un système harmonieux. L'introduction dans ce système d'un corps étranger, même en quantité minime, provoque toujours une réaction plus ou moins énergique de l'organisme tout entier. Ce ne sont pas seulement les cellules libres qui réagissent, mais toutes les autres: les cellules des tissus conjonctifs et réticulo-endothéliaux, des vaisseaux, des organes hématopoïtiques, des nerfs, etc.

Nous pensons que l'immunité consiste essentiellement dans les réactions défensives des cellules qui prennent part à la destruction et à l'expulsion des parasites. Attendu que toutes ces réactions sont obligatoires et involontaires, nous pouvons dire que nous avons affaire à des réflexes. Ces réflexes de défense peuvent changer et varier sous l'action de différents excitants, microbes et toxines qui pénètrent dans l'organisme.

Pour bien comprendre le mécanisme de l'immunité, il faut étudier non seulement les produits des réactions défensives des cellules, produits qu'on trouve dans le sang et les sérums, mais il faut encore et surtout étudier minutieusement la vie et les modifications des cellules elles-mêmes, leur rôle dans la lutte contre les microbes, leur relation entre elles, l'influence des sécrétions internes et le rôle du système nerveux dans l'activité des cellules.

II. FACTEURS DE L'IMMUNITÉ.

Nous avons fait de nombreuses expériences sur les animaux inférieurs et sur les animaux supérieurs.

En comparant les facteurs de l'immunité chez les invertébrés et les vertébrés, on constate qu'il n'y a pas en principe une bien grande différence dans leurs moyens de défense contre les microbes.

La défense de l'organisme se réalise dans les deux cas grâce à l'activité des cellules et se réduit à cinq méthodes principales:

1° *Digestion intracellulaire* ou phagocytose.
2° *Formation des cellules géantes*, sorte de coopération qui renforce le travail des cellules isolées.
3° *Formation des capsules* qui entourent les cellules géantes avec les microbes et les mettent à l'abri des organes et des cellules saines.
4° *Élimination des microbes*. Cette élimination se réalise essentiellement par une formation d'abcès.
5° *Formation des anticorps.*

De ces cinq facteurs, le plus général est la réaction phagocytaire. Elle s'observe du bas en haut de l'échelle des invertébrés et des vertébrés.

Une énorme quantité d'observations établit avec précision l'existence d'un parallélisme parfait entre l'immunité contre quelque infection et les réactions phagocytaires. Le rétablissement est une conséquence de cette réaction. Si cette réaction ne se produit pas, les microbes de la maladie prennent le dessus et l'organisme périt. Si la phagocytose ne se produit pas, l'organisme reste au pouvoir de ses parasites. Et ceci se rapporte non seulement aux invertébrés, mais également aux vertébrés.

Ces faits, établis pour la première fois par Metchnikoff dans ses travaux classiques sur différents invertébrés, conservent leur justesse jusqu'à présent.

La formation de plasmodes ou de cellules géantes est également une réaction des cellules, généralement observée après l'introduction dans l'organisme de substances étrangères.

Ces cellules géantes ou plasmodes commencent, avec le temps, à dégénérer peu à peu et de nouveaux leucocytes arrivent de toutes parts. Ils se disposent autour d'elles en cercles concentriques et forment une capsule de tissu conjonctif à l'intérieur de laquelle les microbes sont définitivement digérés, se transformant en un pigment brun-foncé. Ces processus rappellent beaucoup ce qui se passe chez les animaux supérieurs lors de la formation du tubercule. A la formation de cellules géantes et de capsules prennent part non seulement les phagocytes, mais aussi les autres cellules libres du sang.

Nous avons observé chez les chenilles, dans certains cas, une réaction cellulaire de défense extrêmement curieuse qui rappelait la formation d'un abcès. Si l'on injecte à une chenille une dose assez forte de microbes peu virulents (*B. thirotrix* ou *B. perfringens*) on peut observer quelques jours après des taches noires sur la peau. L'étude de ces taches sur les coupes a montré que nous avons là de grandes agglomérations de leucocytes entourant une masse de microbes en partie digérés et transformés en pigment noir. Ces agglomérations se trouvent immédiatement sous la peau. L'épiderme et la cuticule commencent peu à peu à se pigmenter et à se désagréger à cet endroit. Finalement, le contenu de cet abcès sort à l'extérieur. Nous avons ici un exemple remarquable de réactions cellulaires de défense.

Tout comme au moment de la formation des abcès chez les animaux supérieurs, la couche supérieure des cellules élimine des ferments qui détruisent l'épiderme et préparent l'ouverture par laquelle seront expulsés les microbes et le pus.

En même temps, les leucocytes se trouvant du côté opposé, sous l'abcès, agissent autrement: ils n'éliminent pas de ferments pour la destruction des tissus environnants mais constituent, au contraire, un tissu dense, une barrière, qui empêche la pénétration des microbes à l'intérieur de l'organisme. Comment comprendre et interpréter ce travail si conforme au but, ce travail des cellules libres, qui se trouvent au même endroit, mais qui agissent dans un sens opposé?

Il nous semble que ce phénomène ne peut être interprété qu'en admettant l'idée que les leucocytes se trouvent sous l'influence d'un régulateur qui leur imprime telle ou telle direction.

Il est difficile d'admettre que des millions de cellules différentes, prenant part à la constitution d'une formation aussi rationnelle qu'un abcès, puissent agir, spontanément sans aucun plan, attirées seulement par la chimiotaxie.

Il est possible que l'activité des cellules libres, c'est-à-dire de différents leucocytes, soit réglée par le système nerveux comme celle de toutes les autres cellules et de tous les autres organes.

III. RÔLE DU SYSTÈME NERVEUX DANS L'IMMUNITÉ.

Il est impossible d'admettre que des processus si complexes et si conformes au but que ceux que nous trouvons dans la défense organique pourraient se produire dans l'organisme indépendamment du système nerveux. Mais comment démontrer ce rôle des centres nerveux dans les réactions de l'immunité?

Nous avons fait de nombreuses expériences sur les grenouilles mais elles n'ont pas réussi. Nous nous sommes alors adressés aux chenilles de *Galleria mellonella* qui conviennent bien à ce genre de recherches. Elles s'immunisent très facilement envers différents microbes et elles présentent en outre cet avantage que leurs centres nerveux se trouvent au-dessous des téguments et sont très faciles à détruire par brûlure.

Nous nous sommes servis pour cette opération d'un fil de platine chauffé au rouge. Les chenilles supportent bien cette opération et lui survivent deux semaines. Nos expériences ont démontré que les chenilles sans ganglions cérébraux s'immunisent bien contre les microbes.

Les chenilles privées d'un ou de deux ganglions thoraciques s'immunisent aussi très bien. La destruction du troisième ganglion thoracique diminue rapidement l'immunité naturelle et acquise (Metalnikov, 1927).

La destruction d'un des ganglions abdominaux n'empêche pas l'immunisation de ces insectes. Ces faits prouvent que les nerfs jouent un rôle très important chez les insectes en ce qui concerne l'immunité.

Tout récemment nous avons fait, en collaboration avec Mlle Ermolaeff (1931), des expériences qui démontrent encore plus clairement le rôle du système nerveux dans l'immunité.

Nous laissons d'abord les chenilles jeûner pendant plusieurs jours. Ensuite nous les ligaturons fortement au milieu de leur corps. Les chenilles supportent très bien cette opération et vivent encore deux ou trois semaines.

Les deux parties du corps sont complètement séparées l'une de l'autre. Si nous infectons la partie antérieure, celle-ci meurt en 15–24 heures et la partie postérieure reste vivante pendant encore 2–3 semaines. L'expérience, faite inversement, donne les mêmes résultats.

En utilisant la même technique on immunise facilement l'une ou l'autre partie du corps. Mais, ce qui est surprenant, c'est que l'immunisation de la partie antérieure du corps suffit pour transmettre l'immunité à la partie postérieure qui en est cependant complètement séparée.

Puisque le 3° ganglion thoracique de la partie antérieure se trouve en communication avec les ganglions de la partie postérieure par l'intermédiaire d'une chaîne nerveuse ventrale, il faut admettre que l'immunité de la partie postérieure est transmise à celle-ci par le système nerveux. Les commissures de la chaîne ventrale sont si fines qu'elles ne doivent pas être lésées par la ligature.

Il est beaucoup plus difficile de démontrer le rôle du système nerveux dans l'immunité chez les animaux supérieurs.

Dans une série de travaux faits en collaboration avec C. Toumanoff (1925) et V. Chorine (1926, 1928), nous avons démontré qu'à la base de l'immunité se trouvent les réactions défensives des différentes cellules.

Attendu que toutes ces réactions sont obligatoires et involontaires, nous pouvons dire que nous avons affaire à des réflexes de défense. On est alors appelé à se demander: si l'immunité est le résultat des réactions de défense ou des réflexes, n'est-il pas possible, en se servant de la méthode du Prof. Pavlov, d'obtenir des réflexes conditionnels? Les expériences que nous avons faites en collaboration avec Chorine (1926, 1928) nous ont donné des résultats très démonstratifs.

En associant à une excitation interne (injection de microbes chauffés ou de bouillon dans le péritoine) une excitation externe (grattage ou chauffage 20–30 fois d'une même région de la peau), nous avons pu facilement provoquer chez les cobayes des réflexes conditionnels typiques.

On sait que, chez un animal, l'exsudat péritonéal présente un liquide complètement transparent qui ne contient que très peu de cellules ou de phagocytes.

Presqu'aussitôt après l'injection d'une substance étrangère dans le péritoine, les leucocytes apparaissent en grand nombre. Ce sont les polynucléaires qui parviennent les premiers. Ensuite les monocytes apparaissent en grande quantité pour atteindre le maximum vers le deuxième jour après l'injection. En dernier lieu viennent les lymphocytes.

Les cobayes préparés comme nous l'avons indiqué et ayant subi une excitation externe donnaient les mêmes réactions de défense que s'ils avaient réellement reçu une émulsion de microbes, c'est-à-dire qu'ils présentent la mobilisation des polynucléaires, des monocytes et des lymphocytes. Il est vrai que cette réaction est plus passagère que chez l'animal ayant reçu une injection de microbes, mais elle n'en est pas moins démonstrative.

En continuant nos expériences, nous avons pu provoquer des réflexes conditionnels sur les réactions cellulaires du sang. On sait que chaque injection d'une émulsion microbienne change brusquement la formule leucocytaire du sang. En associant ces injections à une excitation externe (grattage de l'oreille et son d'une trompette) nous avons pu obtenir des réflexes conditionnels typiques (Metalnikov, 1931).

Plusieurs lapins ont reçu des vibrions cholériques chauffés. Chaque injection était accompagnée d'une excitation externe (grattage de l'oreille et son d'une trompette). 7–10 jours après la dernière injection les lapins ont subi les excitations externes habituelles (2–3 fois en une heure). Nous examinions alors à plusieurs reprises le sang de ces lapins. Ces examens nous ont démontré que la quantité des globules blancs, chez les lapins ainsi traités, avait augmenté considérablement 3–5 heures après l'excitation externe.

Enfin, nous avons fait une série d'expériences sur le rôle des réflexes conditionnels dans la formation des anticorps. Plusieurs lapins ont reçu chaque jour (20–30 jours de suite) 2 cm.^{3} d'émulsion de vibrions cholériques chauffés. Chaque injection était précédée d'une excitation externe (grattage ou chauffage d'une région). 12–20 jours après la dernière injection, quand le titre d'agglutination fut abaissé, deux ou trois lapins ont subi l'excitation conditionnelle externe. Les lapins de contrôle sont restés sans excitation. Tandis que chez les lapins de contrôle le titre d'agglutination n'a pas changé, chez les lapins ayant subi l'excitation ce taux a très sensiblement monté.

Toutes les expériences que nous venons d'exposer ont été répétées et nos résultats ont été confirmés par plusieurs autres (Vigodchikoff et Barykine, 1927; Podkopaeff et Saatchian, 1928; Nicolau et Antinesco-Dimitriu, 1929; Palletini, 1929; Ostrorsky, 1930).

Le fait qu'une simple excitation externe peut changer brusquement la formule leucocytaire du sang ou augmenter la production des anticorps, démontre très clairement le rôle joué par le système nerveux dans les réactions de l'immunité.

Nous savons bien à présent, d'après les travaux de Pavlov et de ses élèves, que l'écorce des hémisphères cérébraux joue le rôle principal dans la formation des réflexes conditionnels.

La tache biologique de l'écorce est la signalisation. Grâce à l'existence de l'écorce, l'homme et les animaux peuvent transformer les phénomènes si variés du monde environnant en signaux de telle ou telle activité.

La mise en équilibre de l'organisme avec son milieu gagne ainsi en finesse, exactitude et rapidité. La condition la plus générale de la formation d'un réflexe conditionnel cortical est la coïncidence dans le temps de deux foyers d'excitation: l'un dans l'écorce, l'autre dans une région quelconque du système nerveux, pourvu que ce dernier foyer soit déjà en rapport avec une activité quelconque de l'organisme.

La question se pose tout naturellement: par quel moyen les centres nerveux agissent-ils sur les cellules libres qui jouent un rôle principal dans l'immunité? Ces cellules n'ont aucune connexion avec le système nerveux et cependant elles sont sûrement réglées par les centres nerveux.

Comme nous l'avons vu chez les cobayes à réflexes conditionnels, un simple grattage produit une réaction des cellules libres dans le péritoine. On pourrait bien expliquer ce phénomène par la chimiotaxie positive des microbes et des substances étrangères qu'on introduit dans le péritoine. Mais, dans nos expériences nous n'introduisons rien et cependant les globules viennent dans le péritoine comme s'il y avait quelque chose, et ils disparaissent quand l'excitation est finie. Par quelle force sont-ils poussés? Par quelle force sont-ils guidés quand ils viennent travailler à la construction des capsules, des barrières et des abcès?

Tous ces exemples nous prouvent que les globules blancs ne sont pas autonomes, ne sont pas libres bien qu'ils n'aient pas de connexion directe avec le système nerveux.

Nous devons donc admettre que le système nerveux peut agir à distance par l'intermédiaire de quelque facteur: induction, rayonnement ou hormones.

L'organisme présente un système harmonique idéal où des milliers de petites cellules travaillent ensemble, guidées par les centres nerveux. Il est impossible d'admettre qu'il existe dans ce système quelque catégorie de cellules autonomes pouvant agir indépendamment.

Dans un livre récemment paru, Speransky (1930) donne un résumé de tous ses travaux sur le rôle du système nerveux en pathologie. L'étude de ce rôle dans les divers processus pathologiques lui a permis de constater et de montrer le lien qui existe entre le processus pathologique périphérique et la lésion du système nerveux. Il a pu, en partie, saisir le mécanisme de ce lien. Dans une suite d'ingénieuses expériences et d'observations, il a montré que non seulement le système nerveux est intéressé dans tous les processus pathologiques locaux et généraux, mais que souvent il préside lui-même à leur apparition.

Le livre de Speransky nous donne de plus l'exposé d'un très grand nombre d'expériences montrant le rôle du système nerveux dans les processus pathologiques locaux et généraux ainsi que dans l'immunité.

La théorie des réflexes conditionnels devait nécessairement attirer l'attention des travailleurs sur la parole, qui est un des excitants pouvant provoquer dans l'organisme des réactions défensives bien définies.

Du moment que toute excitation extérieure (chauffage, grattage, son, lumière, etc.) peut devenir un excitant conditionnel, provoquant une réaction définie des glandes salivaires ou d'autres organes, la question se pose naturellement: Le même résultat ne pourrait-il pas être atteint par la parole? Autrement dit, ne pourrait-on pas transformer un mot déterminé en excitant conditionnel?

Le Professeur Platonov (1930), dans un livre extrêmement intéressant qui vient de paraître, expose un grand nombre d'expériences de cet ordre, faites soit par lui-même, soit aux Laboratoires des Professeurs Bechterev, Protopopov et Katkov. Tous ces savants ont réussi à créer des réflexes conditionnels par la parole, intéressant le sommeil, le pouls, la tension artérielle, les organes des sens, le centre de vomissement, etc.

Un intérêt particulier s'attache aux expériences, montrant l'influence d'une excitation verbale sur le système vaso-moteur, sur la fonction trophique et sur les autres fonctions du système nerveux végétatif.

Comme exemple d'un trouble local profond de la circulation produit par la parole, on peut citer les expériences bien connues de Charcot qui réussit à provoquer par la parole l'œdème du bras. Des expériences analogues ont été faites par Weber, Krafft-Ebbing, Sorel et autres.

Toutes ces expériences furent reprises en Russie tout récemment. Le Docteur Finne (1928), en présence d'un groupe de médecins, a provoqué par suggestion verbale de vraies brûlures du 2^e^ degré. Le Docteur Podiapolsky (1904) a pu obtenir, par suggestion verbale, des abcès.

Le Docteur Soumbaieff (1928) a obtenu par le même procédé divers troubles vaso-moteurs: des oscillations de température, des irruptions, des brûlures, etc.

Nous savons, par les observations et par notre propre expérience, combien dans l'état psychique même nos idées, nos représentations et souvenirs agissent sur notre organisme. Un triste évènement, une nouvelle désagréable, la mort d'un ami peuvent provoquer un brusque changement de l'activité du cœur, de la respiration, etc. Il n'est pas rare qu'un simple souvenir, ou même une pensée, une représentation quelconque, provoque chez beaucoup d'hommes une surexcitation des glandes sexuelles.

Si l'état psychique, c'est-à-dire l'état d'âme, agit si fortement sur le cœur et la sphère sexuelle, il n'y a point de raison de supposer qu'elle agisse moins sur les autres organes et les réactions de défense de l'être vivant.

IV. CONCLUSIONS.

De nombreux biologistes et bactériologistes interprètent l'immunité comme une adaptation ou une accoutumance progressive au virus ou à sa toxine. En effet, une telle immunité existe. Les cellules vivantes, les microbes, de même que tout autre organisme vivant, peuvent s'adapter facilement aux différentes conditions défavorables et s'accoutumer aux substances toxiques, surtout si l'adaptation se produit progressivement. Il est bien connu que l'on peut habituer les infusoires et autres organismes à supporter des doses mortelles d'alcool et de diverses substances toxiques. C'est une sorte d'*immunité d'adaptation*. Mais il en existe une autre—*l'immunité de défense*. Cette dernière ne semble présenter que peu de rapport avec l'immunité d'adaptation. On peut même dire qu'elle a pour base des principes tout à fait différents. L'immunité d'adaptation est basée sur la perte de sensibilité de la cellule vivante envers une certaine dose donnée de poison. L'infusoire qui réagissait très fortement à une dose de poison, après l'adaptation ne réagit plus à cette même dose. Il est devenu insensible. *L'immunité de défense* est basée, au contraire, sur l'augmentation de la sensibilité de la cellule, c'est-à-dire sur la faculté qu'ont les cellules de réagir, de lutter plus activement contre les microbes et les parasites ayant pénétré dans l'organisme.

Nous basant sur un très grand nombre de travaux effectués tant sur les vertébrés que sur les invertébrés, nous sommes en droit d'affirmer que toutes les réactions cellulaires deviennent après l'immunisation plus rapides, plus énergiques et plus efficaces. On peut dire que toutes les cellules paraissent devenir plus sensibles envers un microbe donné. De ce point de vue l'immunisation est une mobilisation et une sensibilisation et cette sensibilité renforcée (hypersensibilité) qui semble être la cause principale de l'immunisation et de l'immunité acquise.

La sensibilité est, comme on le sait, la propriété générale de tout organisme vivant. C'est ce critérium qui nous permet de distinguer la vie de la mort. Il n'y a pas de vie ni de processus vitaux sans sensibilité.

La sensibilité est la faculté grâce à laquelle l'organisme réagit à tout excitant. Plus l'organisme est sensible, plus sa réaction est forte.

Grâce à la sensibilité les spermatozoïdes se dirigent vers l'œuf et le fécondent; grâce à la sensibilité les plantes dirigent leurs feuilles vers la lumière et les racines s'enfoncent dans la terre vers les substances nutritives; grâce à la sensibilité les phagocytes se dirigent vers l'endroit infecté, englobent les microbes et produisent un travail très compliqué et final en construisant des cellules géantes des capsules, des abcès, etc.

On peut dire que pendant l'immunisation toutes les cellules se mobilisent et se sensibilisent contre les microbes ou l'antigène donnés comme s'il s'agissait pour elles de combattre un véritable ennemi. Si cet ennemi réapparaît dans l'organisme, les phagocytes se précipitent sur lui avec une grande rapidité. Il se produit une réaction inflammatoire (allergie), une suppuration, un abcès, etc. Plus les cellules sont sensibles, plus elles réagissent activement pour défendre l'organisme.

Actuellement, nous pouvons considérer comme parfaitement prouvé le fait que les cellules sont capables de s'immuniser. Il faut citer les travaux de Petterson (1906) et Salimbeni (1909) qui ont réussi à démontrer que les phagocytes des animaux immunisés, injectés à un animal normal, lui confèrent l'immunité.

D'autre part, nous avons les travaux de Marginesu (1921), Mettermeyer (1924), Carra (1924), et autres qui prouvent que les phagocytes des animaux immunisés possèdent une phagocytose plus forte et plus rapide.

Mais particulièrement probant nous semblent les travaux de Schultz (1910) Friedberger (1911), Dale (1913), et Zinsser (1921, 1923) sur les organes isolés des animaux immunisés et anaphylactisés. Ces auteurs ont fait leurs expériences particulièrement sur l'utérus isolé des cobayes. Ils plaçaient l'utérus dans le sérum artificiel. L'addition d'une petite dose d'antigène provoquait une forte contraction de l'utérus d'un animal immunisé, tandis que l'utérus d'un animal normal ne réagissait pas du tout à des doses beaucoup plus fortes.

Tous ces travaux, ainsi que beaucoup d'autres, que nous ne mentionnerons pas dans cet article, confirment notre hypothèse que dans l'immunisation il se produit une sorte d'hypersensibilité des cellules de l'organisme et même des cellules musculaires.

Mais parfois cette hypersensibilité des cellules est la cause de réactions violentes qui peuvent produire des ébranlements graves et même des chocs mortels. C'est ce que nous observons dans l'anaphylaxie. Si l'antigène est introduit par la voie normale (sous la peau), comme se fait souvent l'infection, il provoque une réaction locale inflammatoire (allergie) qui peut avoir une action bienfaisante, mais si l'antigène est injecté dans le sang ou dans une cavité du corps, il cause une réaction violente qui peut entraîner un choc mortel (Metalnikov, 1922).

Ainsi, à notre point de vue, l'immunité et l'anaphylaxie ont une cause commune: l'augmentation de la sensibilité de toutes les cellules de l'organisme.

Mais quelle est la cause de cette hypersensibilité? Il est bien connu à présent que la sensibilité de toutes les cellules et de tous les organes dépend du système nerveux. La fonction principale des centres nerveux c'est de guider et de diriger toutes les cellules de l'organisme, c'est-à-dire d'agir sur la sensibilité des cellules. Les centres nerveux sont capables de diminuer la sensibilité des cellules, c'est-à-dire de diminuer le dégagement du mouvement nerveux (on désigne ce phénomène sous le nom d'arrêt ou d'inhibition). D'un autre côté, le système nerveux peut occasionner une exaltation de leur excitabilité, c'est-à-dire provoquer une hypersensibilité. Cette action dynamogénique, comme la nomme Brown-Sequard, opposée à l'inhibition, joue également un grand rôle dans la vie de l'organisme et dans sa défense.

C'est ainsi qu'après l'immunisation le système nerveux acquiert une faculté nouvelle: exalter la sensibilité des cellules, c'est-à-dire d'agir dans un sens spécifique contre un antigène donné.

Cette nouvelle faculté se conserve souvent très longtemps. Elle existe encore des mois et même des années après que tous les anticorps ont disparu. Ce n'est que par la mémoire que l'on peut expliquer ce fait que l'immunité, c'est-à-dire la faculté de réagir énergiquement à une excitation spécifique, se conserve très longtemps après que tous les anticorps ont disparu.

A ce point de vue l'immunité présente un problème non seulement biologique et physico-chimique, mais aussi psychologique.

En général, nous ne tenons pas assez compte du rôle que joue le système nerveux, ni de celui de l'action psychique dans la vie de l'organisme. Et cependant il est incontestable que l'affaiblissement des forces psychiques est non seulement la conséquence, mais aussi souvent la cause de diverses affections. A ce point de vue il est regrettable que l'étude de l'organisme soit aussi arriérée. Le rôle des forces psychiques et leur influence sur la vie du corps sont très grands, incomparablement plus grands qu'on ne le pense. Tous les organes: le cœur, les poumons, l'intestin, les glandes à sécrétion interne, sont étroitement liés au système nerveux. Et c'est pourquoi l'état psychique du patient, dans toutes les maladies, a tant d'importance.

Sachant tout cela, nous devons comprendre que dans la lutte contre les maladies, faire œuvre de réaction psychique est tout aussi nécessaire que l'emploi des remèdes.

Par l'éducation de la volonté, par certain entraînement, par l'auto-suggestion ou par la suggestion d'autrui, on peut obtenir des résultats importants.

Il y a nécessité d'élaborer des méthodes spéciales d'éducation, d'exercices, qui développeraient chez l'homme l'empire de la volonté sur son propre corps. Il faut affranchir en quelque sorte l'âme humaine de la dépendance servile du corps. Le maître de l'organisme doit être non le corps, mais son "moi" spirituel, enrichi d'expériences et de science.

A ce point de vue, l'adage "une âme saine dans un corps sain" serait plus justement paraphrasé: "Un corps ne peut être sain qu'avec une âme saine."

Nous avons nombre d'exemples d'individus affectés d'une maladie grave (tuberculose, syphilis) qui vécurent de longues années, atteignant une vieillesse avancée, grâce à une vie sagement vécue. Et combien d'hommes robustes périssent en plein épanouissement de l'âge par suite d'une folle dissipation de leurs forces.

L'homme de ferme volonté sait gouverner ses passions, sa disposition d'esprit, prémunir son corps contre les dangers qui l'entourent et conserver sa vigueur d'esprit jusqu'à un âge fort avancé.

Nous connaissons nombre d'exemples d'hommes ayant atteint une grande longévité qui avaient gardé une âme juvénile, un esprit lucide jusqu'à la dernière minute de leur vie. Sans doute est-ce à une volonté ferme et à une force psychique développée qu'ils devaient d'être restés jeunes jusqu'à la fin de leur vie.

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DIE TEMPERATURVERHÄLTNISSE BEI DEN SOZIALEN HYMENOPTEREN

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(Mit Zehn Abbildungen.)

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I. EINLEITUNG.

DIE Anhäufung von Organismen auf engsten Raum hat immer eine grössere Wärmeentfaltung zur Folge. Wenn die Anhäufung zur Regel wird, wie das bei Insektenstaaten der Fall ist, dann wird die Wärme ein wichtiger Faktor der sozialen Lebensweise. Die Summe der von vielen Einzelwesen erzeugten Wärmemengen beeinflusst in bestimmter Weise den Ablauf der Lebensfunktionen, die als chemisch-physikalische Vorgänge der Van t'Hoff'schen Regel (Reaktionsgeschwindigkeitsregel) unterworfen sind. Um aber einerseits einer gefahrdrohenden Wärmeentwicklung zu begegnen und andererseits die wertvollen Wärmeenergiemengen biologisch auszuwerten, sind regulatorische Massnamen notwendig, die je nach Umfang und Lebensweise der Insektenstaaten sehr verschieden durchgeführt werden. So entwickelte sich bei sozialen Insekten ein Wärmeregulationsvermögen, das mehrere Stufen der Ausbildung umfasst von einfachen Anfängen bis zu erstaunlicher Vollkommenheit. Biologische und physikalische Faktoren mannigfacher Art werden im Insektenstaat zur Erhaltung des notwendigen Wärmegleichgewichtes herangezogen.

Die Insekten werden zu den poikilothermen Tieren (wechselwarmen Tieren) gerechnet. Es soll damit zum Ausdruck gebracht werden, dass ihre Körpertemperatur wenig verschieden von der Umgebungstemperatur ist und mit dieser steigt und fällt.

Diese Begriffstimmung kann jedoch nicht auf alle Insekten ausgedehnt werden, insbesondere nicht auf staatenbildende, deren Nestwärme die Umgebungstemperatur oft um ein Beträchtliches übersteigt. Die Fähigkeit der sozialen Wärmeregulation setzt eine individuelle Wärmeentfaltung voraus, die von der Umgebungstemperatur mehr oder weniger unabhängig ist. Es ist daher zunächst eine Erörterung über die Körpertemperatur staatenbildender Insekten notwendig.

II. DIE BEDEUTUNG DER WÄRME FÜR DAS EINZELWESEN.

Schon frühere Untersucher stellten fest, dass die Körpertemperatur von Insekten, die als gute Flieger gelten, vorübergehend bedeutend höher sein kann, als die Lufttemperatur. M. Girard (1861–63) fand eine Differenz bis zu 10°; in der Brust konnte er immer höhere Temperatur als im Hinterleib messen, was er auf die kräftige Flügel- und Beinmuskulatur zurückführt. Ciesielski (1895) ermittelte in der Brust einer heimkehrenden Biene eine Temperatur von 35°, im Hinterleib dagegen nur 25° C bei einer Lufttemperatur von 13° C. Dieser Umstand und die gleichmässig hohe Nesttemperatur im Bienenvolk verführte Ciesielski zu der Meinung, dass die Bienen zu den warmblütigen Tieren zu zählen seien. Zu dieser in letzter Zeit widerlegten Auffassung bekennt sich auch Brünnich (1922). In seiner umfassenden Untersuchung über die Körperwärme der Insekten erwähnt Bachmetiew (1901) hohe Temperaturen bei Hautflüglern und Schmetterlingen. Verfasser (1925) untersuchte die Körpertemperatur von Bienen, Hummeln und Wespen und kam zu folgenden Ergebnissen: Die Körpertemperatur dieser Insekten ist ausserordentlich wechselnd. In der Ruhe ist sie nur wenig höher als die Umgebungswärme. In der Bewegung kann sie beträchtlich höher steigen. Aber diese Temperaturerhöhung erfolgt nicht nur während einer sichtbaren Bewegung, sondern auch beim scheinbar ruhenden Insekt als Folge eines willkürlich oder reflexmässig ausgelösten Muskeltonus. Äussere Reize wie Kälteeinfluss, Verletzung oder dgl. können zu beträchtlicher Erhöhung der Körpertemperatur führen. Der so erzielte Wärmeüberschuss kann jedoch nur kurze Zeit erhalten werden. Erst nach längerer Pause ist das Insekt in der Lage wieder soviel Wärme zu erzeugen, dass maximale Körpertemperatur erreicht wird. Es können Temperaturdifferenzen bis zu 20° C gemessen werden. Für die Beurteilung des Wärmehaushaltes des Insektenstaates sind gerade diese vorübergehenden Differenzleistungen von Bedeutung. Für die Bienen ergab sich ferner die interessante Feststellung, dass ältere Bienen eine höhere Wärmeleistung aufzuweisen haben als jüngere. 24 bis 48 Stunden alte Bienen hatten eine Durchschnittsdifferenz von nur 1·43° C, Stockbienen im Alter von 3 bis 15 Tagen brachten es auf durchschnittlich 10·2° Temperaturüberschuss, Sammelbienen und Bienen der Fluglochwache im Alter von mehr als 20 Tagen hatten einen solchen von 12·4° bezw. 13·12° C. Ein Unterschied ergab sich auch in der Wärmeleistung der drei Bienenwesen. Die höchsten Temperaturen haben die Drohnen, deren Überschuss durchschnittlich 13·73° C betrug, dann folgen die Arbeitsbienen mit oben angegebenen Wärmeleistungen und zuletzt die Königinnen, bei denen eine Durchschnittsdifferenz von nur 5·72° C gemessen wurde. Da die Bienenkönigin nach dem Hochzeitsfluge den Stock nicht mehr verlässt, verliert ihre Flugmuskulatur sowohl an Arbeits- als auch an Wärmeleistungsfähigkeit. Dass die Körperwärme auch vom Ernährungszustand abhängig ist, haben Messungen an

Hungerbienen ergeben, deren Wärmeleistung ganz erheblich hinter gefütterten Bienen zurückblieb.

Pirsch (1923) hat Körpertemperaturmessungen an Bienen bei steigender Aussentemperatur vorgenommen, die zu folgenden Feststellungen führten:

Lufttemp. (° C)	Körpertemp. (° C)		Durchschnittskörpertemp. (° C)	Unterschied zw. Luft- u. Körpertemp. (° C)	Anzahl der Bienen
	Max.	Min.			
5·5	14	8·5	10·2	4·7	100
21·4	31	22·0	25·8	4·4	100
27·0	30	28·5	29·1	2·1	54
30·5	34	31·5	32·0	1·5	100
35·0	37	34·5	35·1	0·1	100
39·5	42	38·0	39·5	0·0	100
43·5	44	42·5	43·6	0·1	100
52·0	48	45·5	46·0	− 6·0	100
58·0	48	45·5	46·4	− 11·6	11

Diese Zahlen erweisen, dass auch das Einzeltier eine gewisse Regulationsfähigkeit besitzt. In der Nähe der Optimaltemperatur (35° C) stimmen die Temperaturwerte der Umgebung und des Körpers gut überein; bei niedrigerer Aussentemperatur ist die Körperwärme höher, bei überoptimalen Temperaturen dagegen bleibt die Körperwärme zurück. Es findet also in letzterem Falle eine Stoppreaktion statt.

Ein ähnliches Verhalten, allerdings nur in angedeutetem Masse, konnte Verfasser (1927) bei den Bienenlarven feststellen. Im Temperaturbereich von 32–37° C, der dem Entwicklungsoptimum entspricht, ist die Körperwärme der Larven gleich der Umgebungstemperatur (Abb. 1). Zwischen 15° und 32° C ist die Körpertemperatur ca. 0·5° höher, bei Aussentemperaturen von über 37° C ist die Körperwärme niedriger. Anfänge einer Wärmeregulation sind also bereits bei den Bienenlarven unverkennbar. Ganz anders dagegen verhalten sich die Puppen. Bei ihnen ist keine Spur einer Regulation festzustellen. Ihre Körpertemperatur bleibt immer etwas hinter der Umgebungstemperatur zurück, ganz gleich ob diese tiefer oder höher als der Optimalbereich steht. Chemisch-physiologisch ist das Puppenstadium vom Larvenstadium gänzlich verschieden. Etwa vom 7. Larventage an, kurz vor der Verpuppung, schlägt der assimilatorische Stoffwechsel-Prozess in den dissimilatorischen um, eine Nahrungsaufnahme erfolgt nicht mehr bis zum Schlüpfen aus der Zelle. Bei Umgebungstemperaturen unter 15° C verhalten sich die Bienenlarven ähnlich wie die Puppen, die Körpertemperatur ist etwas niedriger als jene der Umgebung, es findet keine Regulation statt. Die Erklärung hiefür dürfte darin zu suchen sein, dass die untere Aktivitätsgrenze für die Brut etwa bei 15° liegt, die Stoffwechselprozesse also auf ein Minimum herabgestimmt sind.

Obwohl die Hummeln an Körpermasse die Honigbiene bedeutend übertreffen, ist ihre Wärmeleistung doch geringer. Sie betrug nach eigenen Messungen z.B. bei *Bombus terrestris* ♀ 7·8° C, bei *Bombus agrorum* ♂ durchschnittlich 10° über der Aussentemperatur.

Bemerkenswert ist der Unterschied in der Wärmeleistung bei den sozialen Faltenwespen. Unsere einheimischen *Vespa*-Arten weisen viel höhere Wärmeüberschüsse auf als die hinsichtlich des Nestbaues und der Brutpflege von ihnen etwas verschiedenen *Polistes*-Arten. Die sehr interessanten Zusammenhänge zwischen Körpertemperatur, Brutwärme und Nestbau sollen im nächsten Abschnitt besprochen werden. Wie bei den Bienen sind auch bei den Wespen die Wärmeleistungen der Jungtiere geringer als bei älteren Individuen. Im ganzen bleibt aber die Wärmeerzeugung der Wespen gegenüber jener der Bienen deutlich zurück, wie aus nachstehender Aufstellung (nach Himmer, 1927) deutlich hervorgeht:

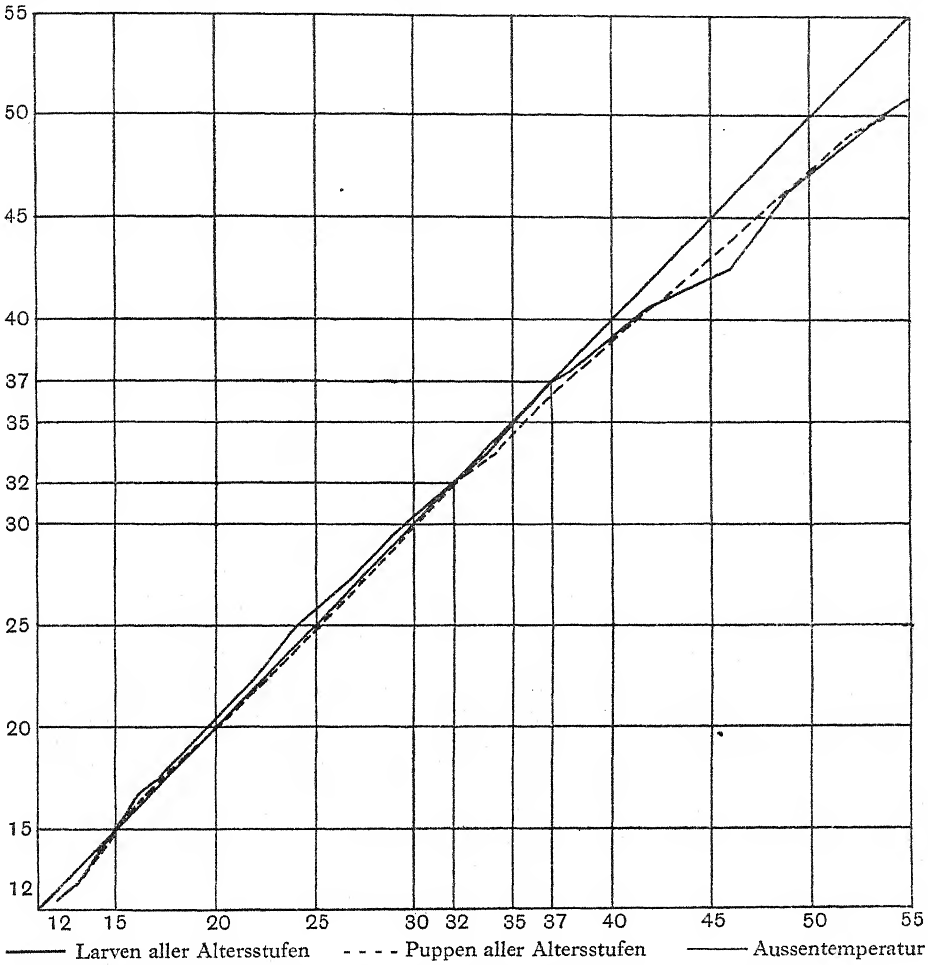

Abb. 1. Kurven der Körpertemperatur von Bienenmaden und -Puppen bei steigender Aussentemperatur.

Bienen (° C)	*Vespa vulgaris* (° C)	*Polistes gallica* (° C)
♂ 13·73	6·24	3·08
♀ 5·72	10·48	
☿ 12·40	7·46	

Auffallend ist die hohe Wärmeleistung der Wespenweibchen verglichen mit jener der Bienenkönigin. Der für die Bienenköniginnen ermittelte Durchschnittswert bezieht sich auf legereife Tiere, die den Stock nicht mehr verlassen und infolgedessen die Flugtüchtigkeit verloren haben. Ihre Muskulatur ist ungeübt und weder zu hohen Arbeitsleistungen noch zu nennenswerten Wärmeleistungen befähigt. Die Wespenweibchen dagegen sind fluggewandte Geschlechtstiere, die sich ihre Nahrung selbst suchen und ausserdem zum Zwecke der Überwinterung reichlich mit Reservestoffen versehen sind. Da sie allein ohne Volksanhang überwintern und den klimatischen Einflüssen viel mehr als die in der Mitte der wärmespendenden Wintertraube sorgsam behütete Bienenkönigin ausgesetzt sind, ist für die Wespenweibchen die Fähigkeit der Wärmeentfaltung von besonderer biologischer Bedeutung.

Eine bestimmende Rolle für den sozialen Wärmehaushalt der Hymenopteren spielt die zur Entwicklung der Brut erforderliche Temperatur. Das Entwicklungsoptimum für die sozialen Insekten unserer geographischen Breiten liegt durchwegs über dem klimatischen Temperaturmittel. Bei der Honigbiene fällt das Optimum der Entwicklung beinahe zusammen mit den vitalen Temperaturgrenzen, während bei Wespen und Ameisen dem vitalen Temperaturbereich ein weit grösserer Spielraum zur Verfügung steht. Leider beschränken sich die Angaben über die Bruttemperaturen der sozialen Hautflügler nur auf wenige Arten. Über das Entwicklungsoptimum der Ameisen teilt Steiner (1924, 1925, 1929) nachstehende Werte mit:

Formica fusca	... ...	35–36° C
F. rufa ...	... ...	23–29° C
Lasius flavus	... ...	23–28° C
L. niger ...	obere Optimalgrenze	31° C
Myrmica rubra ruginodis	obere Optimalgrenze	31° C
Tetramorium caespitum	obere Optimalgrenze	31° C

Von sozialen Faltenwespen sind die Entwicklungsoptima folgender Arten bekannt:

Polistes gallica ...	... ...	im Mittel 35·5° C (Steiner)
Vespa vulgaris ...	... ...	29·5–32° C (Himmer)
V. crabro	... ...	29·8–31·8° C (Himmer)

Diese Angaben beziehen sich auf die speziell untersuchten Fälle. Dass sich diese Werte unter anderen Umweltbedingungen etwas verschieben können, ist als naturgemäss anzunehmen. Im übrigen erleidet die Entwicklung der Ameisen- und Wespenbrut auch bei unteroptimalen Temperaturen keine Unterbrechung. Die Brut kann sogar ohne Schaden eine erhebliche Unterkühlung vorübergehend ertragen.

Etwas abweichend davon ist das Verhalten bei den Entwicklungsstadien der Honigbiene. Verfasser (1927) stellte ein Entwicklungsoptimum im Bereiche von 34·5–35° C fest. Die vitalen Grenzen liegen zwischen 32° und 36° C. Bei tieferen Temperaturen schlüpfen—soweit sie überhaupt noch zur Ausbildung kommen—

Imagines mit verkürzten oder verkümmerten Körperanhängen (Abb. 2), wobei besonders die Flügel erhebliche Stauchungen aufweisen können. Solche Missbildungen sind natürlich nicht lebensfähig. Höhere Temperaturen als 36° C wirken bereits tötlich. Diese für die Existenz der Brut so kleine Temperaturspanne gibt dem sozialen Wärmehaushalt des Bienenstaates sein besonderes Gepräge.

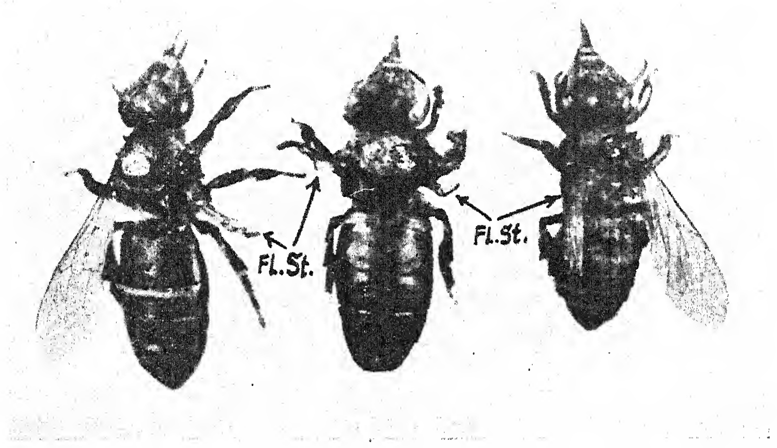

Abb. 2. Bienen mit verkümmerten Flügeln. Die Bienen sind bei unteroptimaler Temperatur geschlüpft. *Fl.St.*, Flügelstummel.

Auch in Bezug auf den Zusammenhang zwischen Aktivität und Temperatur unterscheidet sich die Honigbiene in typischer Weise von den anderen sozialen Hymenopteren. Während nach Beobachtungen von Steiner (1924) Ameisen noch bei einer Temperatur von 0–2½° C eine zum Herumkriechen ausreichende Beweglichkeit besitzen und auch die Wespen bei Temperaturen von wenig über 0° ihre Beweglichkeit noch nicht ganz verloren haben, verfällt die Biene als Einzeltier bereits bei 6–7° C in vollständige Kältestarre. Bei 13° C ist die Bewegungsfähigkeit schon bedeutend vermindert. Die Honigbiene ist somit weit mehr auf Wärme als ihre nächsten Verwandten angewiesen.

Die Fähigkeit der Wärmeregulation setzt zwangsmässig einen hoch entwickelten Temperatursinn voraus. Schon Forel (1920) fand bei Ameisen ein feines Unterscheidungsvermögen für Temperaturunterschiede und Herter (1924) hat bei *Formica rufa* eine Temperaturempfindlichkeit für Abweichungen von ½° C nachgewiesen. Das Temperaturoptimum (Vorzugstemperatur) ist jedoch bei den Ameisen nach Herter nicht konstant, sondern ist abhängig von Licht und Durchschnittshöhe der Aussentemperatur, nicht aber von der Feuchtigkeit. Über den Temperatursinn der Wespen und Bienen liegen zwar bis heute noch keine besonderen Untersuchungen vor, doch aus der Art und Weise ihres sozialen Wärmehaushaltes geht ohne Zweifel hervor, dass ihr Unterscheidungsvermögen für Wärmeabstufungen mindestens ebenso gut ausgebildet ist, wie das der Ameisen.

III. DER SOZIALE WÄRMEHAUSHALT.

Der Wärmehaushalt der staatenbildenden Hautflügler weist verschiedene Typen auf. Er passt sich einerseits der biologischen Eigenart des Einzelwesens sowie der Gesamtkolonie, andererseits den physikalischen Bedingungen der Umgebung an. Die im vorhergehenden Abschnitt näher beschriebene Fähigkeit der Erzeugung eines Wärmeüberschusses und der Wärmeunterscheidung (Temperatursinn) treten in den Dienst der Wärmeregulation. Dazu kommen noch verschiedene biologische und physikalische Faktoren wie z.B. Anlage eines schutzbietenden Nestbaues, zweckmässige Ausnützung klimatischer Einflüsse, Ventilation, Wassertransport, Brutverschiebung, Erzeugung von Muskelwärme, Transpiration.

Für die Auswahl dieser Regulatoren sind massgebend die Wärmeansprüche der Brut und der Imagines, Höhe und Grenzen des Entwicklungsoptimums, Art der Überwinterung, Grösse der Kolonien und Klimacharakter.

(1) DER WÄRMEHAUSHALT DER HONIGBIENE.

Bei der Honigbiene ist zu unterscheiden zwischen dem thermischen Zustand des brütenden Sommervolkes und dem des brutfreien Wintervolkes. Die zahlreichen früheren Messungen über den Wärmeverlauf im Bienenstock haben kein klares, einheitliches Bild des Wärmehaushaltes gegeben. Die Beobachtungen erstreckten sich entweder auf eine zu kurze Zeit oder sie wurden in zu grossen Zeitabständen vorgenommen. Vielfach waren die ermittelten Temperaturwerte nicht der Ausdruck normaler Vorgänge sondern durch mechanische Störung des sehr empfindlichen Bienenvolkes hervorgerufene Reiztemperaturen, die nur bei grösster Vorsicht zu vermeiden sind.

Die ersten systematischen Dauermessungen führte Gates (1914) aus. Er benützte fünf Quecksilberthermometer und nahm die Messungen stündlich mit Ausnahme der Nachtzeiten während eines ganzen Jahres vor. Mehrmals wurde ununterbrochen 2–3 Tage lang abgelesen.

Das bemerkenswerteste Ergebnis seiner Beobachtungen ist, dass in der Mitte des zu einer engen Traube zusammengeschlossenen brutfreien Wintervolkes zeitweise ein Temperaturverlauf herrscht, der entgegengesetzt zur Aussentemperatur steht. Bei grosser Kälte im Freien zeigte das Traubenthermometer hohe Temperaturen an, bei zunehmender Umgebungswärme ging die Traubentemperatur zurück. Die täglichen Schwankungen betrugen höchstens 6° C. Im allgemeinen fiel die Temperatur nicht unter 20° C. Der tiefste Stand betrug 17° C. Das winterruhende Volk ist gegen mechanische Störungen sehr empfindlich und reagiert darauf mit sofortigem Temperaturanstieg. Die Grösse der Bienentraube wechselt ständig. Bei warmer Witterung lockert sich die Traube, bei Kälte zieht sie sich zusammen. Die Ausdehnung erfolgt gewöhnlich nach unten in der Richtung auf das Flugloch. Zwischen den Bienen der Aussenschicht und den Innenbienen findet ein ständiger Austausch statt. Die Randbienen sind weniger beweglich und sitzen alle eng zusammengepresst mit den Köpfchen nach innen und dem Hinterleib nach aussen gerichtet.

Phillips und Demuth (1914), welche sich für ihre Messungen der thermoelektrischen Methode mit Fernablesung bedienten und daher jeden Anlass einer Störung vermeiden konnten, kamen im Wesentlichen zu ähnlichen Ergebnissen, die sie in folgenden Sätzen (zit. nach Armbruster, 1923) zusammenfassen: "Wenn die Temperatur eines nicht gestörten Volkes ohne Brut zwischen 14° und 20·5° schwankt, dann verhalten sich die Bienen ruhig. Ihre Temperatur wechselt mit der draussen. Bei niedrigen Temperaturen bilden sie eine feste Kugel und die Temperatur im Kugelinnern steigert sich durch die von den Bienen selbst erzeugte Wärme."

Weiter beobachteten Phillips und Demuth dass die Aussenschicht der Bienentraube aus fast bewegungslosen, eng aneinander sitzenden Bienen besteht, während der innere Kern locker gefügt ist und die Bienen dieser Zone sich lebhaft bewegen, mit den Flügeln fächeln und hin- und herkrabbeln. Je unruhiger sie sich verhalten, desto mehr steigert sich die Wärme. Auffallend sind die Angaben über die untere Grenze der Traubentemperatur von 14° C, die als normal bezeichnet wird, während Gates als untere Grenze 20° ermittelte. Die Messungen späterer Untersucher stimmen eher mit den Ergebnissen von Gates überein.

Einen abweichenden Standpunkt von der bisherigen Auffassung über den Wärmehaushalt im brutlosen Bienenvolk nimmt Armbruster (1922, 1923, 1924) ein. Auf Grund von älteren Messungen eines deutschen Bienenzüchters namens Lammert, die später durch eigene Beobachtungen ergänzt wurden, kommt Armbruster zu einer Wärmetheorie, die er in mehreren Veröffentlichungen vertritt. Die von Lammert aufgezeichneten Wärmekurven sind das Ergebnis von Messungen, die mit Hilfe von Quecksilberthermometern in halbstündigen Ablesungen Tag und Nacht hauptsächlich in der Zeit vom 18. Januar bis 15. Februar 1896 durchgeführt wurden. Ausserdem stand noch eine Jahreskurve zur Verfügung, die sich aus täglich dreimal wiederholten Ablesungen ergab. Armbruster findet hiefür folgende Auslegung: Die Temperatur in der winterlichen Bienentraube bewegt sich unbeeinflusst von der Umgebungswärme in zeitlich rhythmischem Wechsel durchschnittlich in einem Temperaturabschnitt zwischen 13 und 25° C. Die Minimaltemperatur von 13° ist eine Reiztemperatur (Kritischer Punkt), bei der die Bienen unruhig werden, ihren Zusammenhang lockern, Nahrung aufnehmen und durch Muskel- und Atembewegungen Wärme erzeugen. Es kommt somit zu einem sogenannten "Heizsprung," die Temperatur steigt rasch innerhalb einer Stunde auf ca. 25°. Dann hört die Wärmeerzeugung auf, die Temperatur fällt wieder und zwar anfangs etwas rascher, die Bienen ziehen sich enger zusammen, die Temperatur fällt infolgedessen verlangsamt weiter bis zum kritischen Punkt von 13° C. Der Kühlfall dauert 21 Stunden; der ganze Vorgang beginnend bei der kritischen Temperatur, "Heizzeit," "Kühlzeit" bis zur nächsten kritischen Temperatur nimmt also 22 Stunden in Anspruch. Die Hautbienen wechseln dauernd ihren Platz; um sich vor Erstarrung zu schützen, tauchen sie in die wärmere Schicht der Traube ein und überlassen anderen den Aussenplatz. Der Austausch der Hautbienen geht mit zunehmender Abkühlung immer rascher vor sich bis schliesslich bei Erreichung der kritischen Temperatur im Traubeninnern eine allgemeine Unruhe einsetzt und damit eine neue Heizperiode beginnt.

Diese Darstellung des Wärmehaushaltes im Bienenvolk hat in wissenschaftlichen Kreisen mit Recht Widerspruch gefunden, da sie ganz im Gegensatz zu jeglicher Erfahrung über den Einfluss der Umwelt auf innere Lebensvorgänge besonders bei niederen Tieren steht. Unerklärlich ist ein Rhythmus von 22 Stunden, der sich nicht mit dem Ablauf der Tageszeiten deckt, wovon doch im allgemeinen die Aussentemperatur und von dieser wieder die Wärme im Innern des Bienenkastens im bienenfreien Raum abhängig ist. Die Luft aber, die die Bienentraube umspült, beeinflusst ohne Zweifel die Wärmeverhältnisse im Innern der Traube. Man würde daher eher einen Wärmeverlauf erwarten, der auf eine 24 stündige Periode eingestellt ist, wenn nicht innere und äussere Faktoren einen derartigen regelmässigen Temperaturgang unterbrechen würden. Ausser den Kurven hat Lammert keinerlei Aufschreibungen über die näheren Umstände seiner Beobachtungen hinterlassen. Es bleibt daher die Vermutung bestehen, dass irgend welche störenden Nebeneinflüsse die Bienen zu regelmässigen Heizaktionen veranlasst haben. Es ist auch wenig wahrscheinlich, dass die Messinstrumente richtig in der Mitte der Bienentraube untergebracht waren, sonst wäre ein Temperaturtiefstand von 13° C wohl kaum erreicht worden.

Ungefähr gleichzeitig aber unabhängig von einander haben Verfasser (1926) in Deutschland und Hess (1926) in der Schweiz eingehende Messungen an Bienenvölkern unter Zugrundelegung der bisherigen Erfahrungen vorgenommen. Um Ablesefehler und Störungen, die bei der Verwendung von Quecksilberthermometern unvermeidlich sind, auszuschliessen, wurden Widerstandsthermometer bezw. Thermoelemente eingeführt, die mit Registriervorrichtungen verbunden waren. Die Aufzeichnungen erfolgten somit ohne jede zeitliche Unterbrechung ganz automatisch und konnten auf einen beliebigen Zeitraum ausgedehnt werden. Die Untersuchungen des Verfassers erstreckten sich auf einen Zeitraum von vier Jahren und wurden an mehreren Bienenvölkern in verschiedenen Bienenwohnungen und unter verschiedenen Umgebungsverhältnissen, im Freien, im Bienenhaus und im Bienenkeller durchgeführt. Hess lötete die Thermoelemente in die Mittelwände der Waben ein, um jede unmittelbare Berührung mit den Bienen zu vermeiden. Durch Einführen von 27 Messtellen, die im ganzen Bienenkasten verteilt waren, konnte er besonders schön die Wärmetopographie des Bienenvolkes ermitteln.

Die Ergebnisse der beiden Untersuchungen stimmen in den wesentlichen Punkten überein, so dass sie als gesicherte Tatsachen gewertet werden können. Die Befunde von Gates sowie von Phillips und Demuth werden im grossen Ganzen bestätigt. Darüber hinaus werden noch weitere wichtige Tatsachen festgestellt, die nunmehr ein klares Bild vom Wärmehaushalt im winterruhenden, brutlosen Bienenvolk geben.

In der Bienentraube herrschen bestimmte thermische Gesetzmässigkeiten. Die grösste Wärme findet sich in der Traubenmitte, von hier fällt die Temperatur gegen die Aussenschicht mehr oder weniger steil ab. Von allen Punkten der Bienentraube ist das Wärmezentrum den grössten Temperaturschwankungen unterworfen, die eine deutliche Abhängigkeit vom Verlauf der Aussentemperatur zeigen (Abb. 3). Wenn letztere einen periodischen Charakter annimmt, dann schwankt auch die

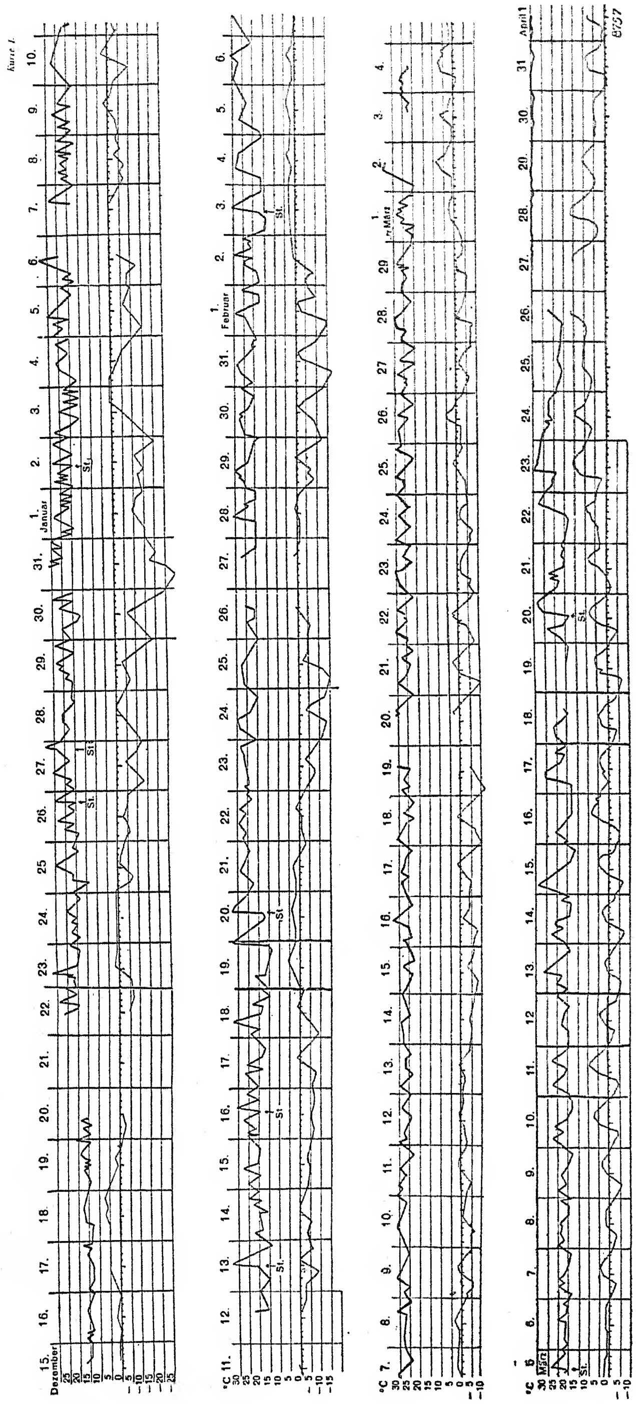

Abb. 3. Temperaturverlauf in der Traubenmitte eines überwinternden Volkes in der Zeit vom 15. Dezember bis 1. April. Obere Kurve, Traubentemperatur; untere Kurve, Aussentemperatur. (Aus Himmer.)

Traubentemperatur im gleichmässigen Rhythmus, falls sich nicht andere störende Einflüsse geltend machen. Ausserhalb des Wärmemittelpunktes gegen den Traubenrand zu verflachen die Temperaturschwankungen und hören an der äussersten Schicht fast ganz auf. Die Temperatur schwankt im Innern etwa im Bereiche von 15 bis 30°, nach Hess zwischen 20 und 32° C. Solche Beobachtungsunterschiede mögen in der innern Verfassung der Völker oder auch in der Art der Umgebungseinflüsse liegen. Hess stellte als grösste Differenz zwischen der Temperatur in der Traubenmitte und in der Umgebung 43° fest, nämlich + 32° Traubentemperatur bei – 11° Aussentemperatur. Verfasser ermittelte im strengen Winter 1923–24 mehrmals Differenzen von über 50° C, einmal wurden im Traubeninnern + 31°, im Freien – 28° C gemessen, somit ein Unterschied von 59° C gefunden. Diese ungewöhnliche kalorische Leistung gibt eine Vorstellung von der Fähigkeit der Wärmeregulation der Bienen.

Während der eigentlichen Winterzeit, besonders bei Frostwetter zeigt sich, wie die amerikanischen Beobachter bereits feststellten, eine auffallende Gegensätzlichkeit im Temperaturverlauf in der Traube und in der Umgebung: bei tiefer Aussentemperatur hohe Innentemperatur und umgekehrt. Die Reaktion der Bienentraube auf die Veränderung der Aussentemperatur erfolgt allerdings nicht synchron, sondern mit einer Verzögerung bis zu mehreren Stunden. Das ist verständlich, wenn man überlegt, dass sich die äusseren Wärmeschwankungen erst allmählich innerhalb des Bienenkastens auswirken. Diese Verzögerung ist umso grösser je besser der Wärmeschutz der Kastenwände ist, ausserdem haben Luftbewegung und Feuchtigkeitsgrad der Umgebung einen entsprechenden Einfluss.

Die Beziehungen zwischen Trauben- und Aussentemperatur sind jedoch nicht immer dieser Art. Sie erhalten einen anderen Charakter und schlagen in das Gegenteil um, wenn die Durchschnittshöhe der Umgebungstemperatur über eine gewisse Grenze hinausgeht. Die Traubentemperatur verläuft dann nicht mehr im entgegengesetzten sondern im gleichen Sinne mit der Aussentemperatur. Den Temperaturanstiegen und -Abstiegen in der Umgebung folgen die gleichartigen Wärmebewegungen in der Bienentraube (ab 19. März bis 26. März der Abb. 3). Dieser Umschlag im Charakter des Wärmehaushaltes ist bemerkenswert. Er bedeutet, dass der Kampf gegen die Kälte zu Ende ist, dass die Umgebungstemperatur nunmehr jene Grenze überschritten hat, unterhalb der noch Abwehrmassnahmen notwendig sind. Diese Grenze konnte Verfasser im Bereiche von etwa 7–8° C im Freien feststellen. Es sei daran erinnert, dass die Aktivitätsgrenze der erwachsenen Bienen ungefähr auf der gleichen Höhe liegt, dass also Bienen, deren Körpertemperatur so weit abgekühlt wird, in Kältestarre verfallen. Erstarrte Bienen sind aber nicht mehr in der Lage, sich an der Wintertraube festzuklammern, sie fallen ab und gehen—von der Wintertraube losgelöst—zugrunde. Es darf daher am Rande der Wintertraube niemals zu Temperaturen kommen, die das motorische Minimum unterschreiten. Tatsächlich behält die äusserste Randschicht der Bienentraube eine ziemlich gleichbleibende Temperatur von ungefähr 9° C (nach Himmer), nach Hess von 8° C. Damit findet nun das thermische Verhalten der Traubenmitte eine naturgemässe Erklärung. *Die zur Aussentemperatur entgegengesetzte Wärme-*

bewegung im Traubeninnern ist dahin gerichtet, am Traubenrand eine Schwellentemperatur zu halten, die noch diesseits der Aktivitätsgrenze steht. Es handelt sich also um eine typische Wärmeregulation einer Tiergemeinschaft als biologische Einheit. Die Gegenprobe bildet der oben erwähnte Fall bei Umgebungstemperaturen von mehr als 7° C. Bei dieser Wärme ist eine Erstarrungsgefahr für die Randbienen nicht mehr vorhanden, die Regulation ist überflüssig, Trauben- und Aussentemperaturen nähern sich auf einen geringen Abstand und bewegen sich dann parallel.

Mit den Temperaturschwankungen der Umgebung steht die Änderung des Traubenumfangs in Zusammenhang. Bei zunehmender Kälte zieht sich die Traube immer enger zusammen, bei Erwärmung dehnt sie sich aus. Eine Lockerung erfolgt auch gelegentlich der Heizaktionen. Dabei ist zu beobachten, dass der Traubenrand immer dem Temperaturgürtel von 9° C folgt, der je nach der Aussentemperatur bezw. je nach der Wärmeentwicklung in der Bienentraube vom Wärmezentrum mehr oder weniger weit entfernt ist. Wenn die Temperatur im Freien die Grenze von 8° C erreicht oder überschritten hat, dann lockert sich die Traube bis zum Flugloch des Bienenkastens und damit beginnen die ersten Ausflüge der Bienen (Hess, 1926). Diese Phase des Wärmehaushaltes im überwinternden Bienenvolk hält bei mildem Frühjahrswetter bis zum Beginn der Bruttätigkeit an. Sobald aber die Königin mit der Eiablage einsetzt, ändert sich der thermische Zustand im Brutbezirk vollständig. Die Temperatur steigt bis zur Höhe des Entwicklungsoptimums (35° C) und bleibt dann gleichmässig auf dieser Höhe ohne Rücksicht auf die Schwankungen der Aussentemperatur. Vom Wärmehaushalt des brütenden Sommervolkes soll weiter unten die Rede sein.

Eine andere eigenartige Erscheinung stellte Hess im brutfreien überwinternden Bienenvolk fest. Innerhalb des Bienenhaufens ist die Wärme entgegen der physikalischen Regel von oben nach unten geschichtet, die wärmeren Partien befinden sich also, wenn man vom Wärmezentrum absieht, unten. Ausserhalb der Bienentraube dagegen sind die oberen Luftschichten wärmer als die unteren. Das Temperaturgefälle vom Wärmemittelpunkt aus ist daher nach oben wesentlich steiler als nach unten. Die tiefste Temperatur, bei der diese inverse Temperaturschichtung noch gefunden wurde, liegt zwischen 7 und 8° C. Daraus ist zu schliessen, dass sich die Bienen nicht weiter vom Wärmemittelpunkt entfernen als bis zur Isotherme von etwa 8° C. Dieses Verhalten bestätigt in hübscher Weise die bereits oben erwähnte Beobachtung von der Schwellentemperatur am Rande der Wintertraube. Hess erklärt sich die umgekehrte Temperaturschichtung in der Bienentraube damit, dass die Bienen die warme Atemluft nach unten abstossen und die kühlere Einatmungsluft von oben nachsaugen. Das bedeute gleichzeitig eine Ökonomisierung der Wärmeenergie; die frische Luft, die unten beim Flugloch hereinströmt, wird durch die von den Kastenwänden und Waben abgegebene Wärme temperiert, steigt nach oben und ihr Wärmeinhalt kommt den Bienen beim Einatmen zugute. Zwangloser lässt sich nach Himmer die Temperaturschichtung im Bienenhaufen damit erklären, dass die Bienen im unteren Teil der Traube, wo sich die Umgebungseinflüsse wie Licht und Kälte am stärksten geltend machen, in einem erhöhten Reizzustand

(Kälteabwehr) befinden und somit mehr Wärme erzeugen. Ausserdem bewirkt die physikalische Temperaturschichtung ausserhalb der Bienentraube entsprechend der Steigerung des Kälteeinflusses eine nach unten zunehmende Reaktion der auf Wärmeausgleich hinzielenden Abwehrkräfte des Bienenvolkes.

Der Wärmehaushalt des überwinternden Bienenvolkes findet auch im Nahrungsverbrauch einen sinnfälligen Ausdruck. An den Wintersitz schliessen sich die während der Sommermonate gesammelten und im Stock aufgespeicherten Honigvorräte an. Der grösstenteils aus Zucker (Traubenzucker und Fruchtzucker) bestehende Honig ist ein vorzüglicher Wärmelieferant. Eingehende Untersuchungen des Verfassers (1926) haben ergeben, dass die Zehrung eines Bienenvolkes während der Winterzeit umso grösser ist, je mehr die Kälteeinflüsse zur Wärmeerzeugung zwingen. Die Abbildung 4 stellt den Nahrungsverbrauch zweier gleich starker Bienenvölker während der brutlosen Zeit dar. Das eine Volk (ausgezogene Kurve) war im Freien aufgestellt bei wechselnder Aussentemperatur, das andere Volk (punktierte Kurve) war in einem dunklen gut isolierten Kellerraum untergebracht bei einer gleichmässig ansteigenden, schwankungslosen Temperatur von 3–6° C. Einer Gesamtabnahme von 4250 g beim Volk im Freien steht beim Kellervolk eine Gewichtsminderung von nur 1950 g gegenüber, was weniger als die Hälfte ausmacht. Abbildung 5 veranschaulicht die Winterzehrung zweier Völker, die beide in Bienenkästen gleicher Bauart untergebracht waren, nur mit dem Unterschied,

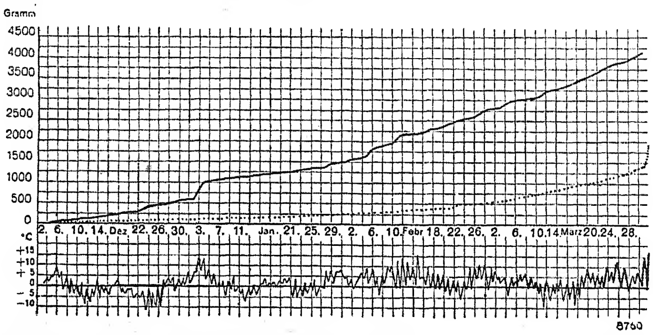

Abb. 4. Nahrungsverbrauch zweier überwinternden Bienenvölker. Volk im Freien = ausgezogene Kurve, Volk im Keller = punktierte Kurve, darunter Kurve der Aussentemperatur. (Nach Himmer.)

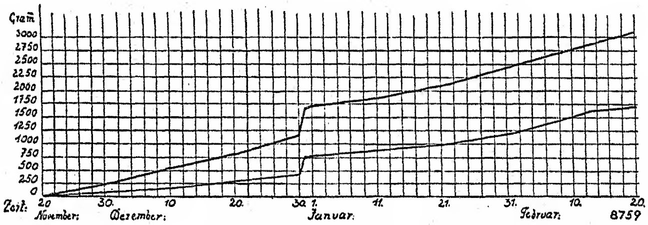

Abb. 5. Winterzehrung zweier gleich starker Völker in verschieden isolierten Kästen. Obere Kurve = Zehrung bei gewöhnlicher Isolierung, untere Kurve = Zehrung bei starker Isolierung. (Aus Himmer.)

dass die Wände des einen Kastens in der üblichen Weise mit einer Isolierschicht aus Holzmehl versehen, der andere aber allseitig mit einer 6 cm starken Wandfüllung von Torfoleum (gepresster Torf) umgeben war. Die Zehrung betrug im ersten Falle 3050 g, im gut isolierten Kasten, der einen weit besseren Wärmeschutz bot, dagegen nur 1700 g. Ähnliche Erfahrungen wurden häufig schon von Bienenzüchtern mitgeteilt. Es hat sich daher in kalten Ländern wie Russland und Nordamerika die Praxis eingeführt, die Bienenvölker nicht im Freien, sondern in frostfreien Räumen oder Kellern zu überwintern.

Die Beurteilung des Wärmehaushaltes im brütenden Bienenvolk ist wesentlich einfacher als im brutlosen. Der Wärmeverlauf wird durch die Wärmeansprüche der Brut bestimmt, deren normale Entwicklung nur innerhalb eines eng begrenzten Temperaturbereiches möglich ist. Wie schon mehrfach erwähnt, liegt die beste Entwicklungstemperatur zwischen 34 und 35° C. Bereits frühere Beobachter haben im Brutnest der Honigbiene eine konstante Temperatur von 35° C ermittelt und neuerdings haben Hess (1926) und der Verfasser (1926) in Dauermessungen diese Bruttemperatur bestätigt gefunden. Hess gibt an, dass für den einzelnen Messpunkt die Temperatur im Tagesverlauf nur um 0·2–0·4° C schwankt. Verfasser fand für eine Zeit von vier Wochen einen mittleren Schwankungswert von 0·55° C. Abweichungen bis zu 34° nach unten oder 36° nach oben können vorkommen, bilden aber die Ausnahme. Im Frühjahre bei Brutbeginn, d.i. in Deutschland im allgemeinen anfangs März, erreicht die Bruttemperatur zeitweilig die untere Grenze von 34°, da die Aussentemperatur häufig noch sehr tief liegt. An ungewöhnlich heissen Tagen des Hochsommers oder infolge eines besonderen Anlasses, wie z.B. einer groben mechanischen Störung (Erschütterung) des Volkes, Fütterung oder dgl., kann die Temperatur kurze Zeit bis 36° steigen. Noch höhere Temperaturen sind äusserst selten und immer die Folge eines groben Eingriffes. Dass das Bienenvolk die Fähigkeit hat, unter Umständen nicht nur die untere, sondern auch die obere Optimalgrenze einzuhalten, das Brutnest also vor schädlicher Überhitzung zu schützen, geht aus einem hübschen Versuch von Hess hervor. Er legte einem Bienenvolk ein Heizkissen unter und trieb die Temperatur im Stock auf 40° C. Innerhalb des Brutnestes stieg die Temperatur jedoch nicht über 36°. Die Bienen haben die Temperatur abgestoppt, was umso bemerkenswerter ist, als auch die Luft in der Umgebung des Flugloches stark erhitzt war. Hess vermutet, dass sie sich des Mittels der Wasserverdampfung bedienten, da sie an heissen Tagen sehr viel Wasser in den Stock tragen.

Von verschiedener Seite wird die Meinung vertreten, dass an der Wärmeerzeugung und Wärmeregulierung im brütenden Bienenvolk die Brut selbst hervorragend beteiligt sei. Die oben mitgeteilten Feststellungen über die Eigentemperatur der Bienenmaden und Puppen geben dieser Ansicht keine Stütze. Der geringe Wärmeüberschuss der Maden bei unteroptimalen Umgebungstemperaturen kommt praktisch nicht in Frage. Verfasser (1927, *a*) hat weitere Versuche angestellt, indem er bienenfreie Waben sowohl mit offener Brut (Larven) als auch mit gedeckelter Brut (Puppen) in einen geschlossenen Kasten überführte und den Wärmeverlust mit dem einer brutfreien Wabe unter sonst gleichen Umständen verglich. Es ergab sich kein

Unterschied; in beiden Fällen erfolgte Abkühlung bis zur Höhe der Aussentemperatur in der gleichen Zeit. Die Brutmasse ist somit nicht imstande, auch nur für kurze Zeit die für die Weiterentwicklung und für die Existenz notwendige Temperatur zu halten. Auch die Drohnenbrut vermag keine höhere Temperatur zu erzeugen. Bruman und Liechti (1929) fanden im Gegenteil, dass die Drohnenbrut durchschnittlich um 0·3° C kühler ist als die übrige Brut. Im Gesamtwärmehaushalt des Bienenvolkes kommt die Brut jedoch derart zur Geltung, dass sie als wärmespeichernde Masse den Wärmeausgleich unterstützt. Da sie nach Strauss (1911) aus ungefähr 90 % Wasser besteht, dürfte ihre spezifische Wärme nahe an 1 heranreichen. Das gleiche gilt für die Honigvorräte und den Wabenbau. Die spezifische Wärme des Honigs beträgt 0·44, des Wachses nach Person 0·82. Die erwachsenen Bienen, besonders die älteren sind somit allein die thermisch aktiven, während Brut, Wabenbau und Vorräte thermisch passive Faktoren des Wärmehaushaltes im Bienenvolk sind.

(2) DER WÄRMEHAUSHALT DER WESPEN.

Die Wespen unterscheiden sich biologisch in mancher Beziehung von den Bienen. Soweit sie staatenbildend sind, treten die Staaten nur während des Sommers auf. Im Herbst gehen alle Nestinsassen bis auf die begatteten Weibchen ein, die einzeln im Schutze von Moos, Erde u. dgl. überwintern, um im Frühjahre ein neues Nest zu bauen. Es besteht somit für die Wespen keine Veranlassung Vorräte zu speichern wie die Bienen. Die Ernährung der Wespen ist nicht so einseitig auf Kohlenhydrate eingestellt, ihre Nahrung ist pflanzlicher und tierischer Herkunft und bedingt eine andere Art des Stoffwechsels. Das Wespennest hat mit dem Bienenstock die Wabenform gemeinsam. Die Waben sind jedoch nur einseitig mit Zellen versehen und horizontal angeordnet, das Baumaterial ist nicht Wachs sondern Holzfaserstoff. Der Wabenbau ist bei den grösseren einheimischen Wespenarten mit einer mehrschichtigen Hülle umgeben. Die Wärmeansprüche der Brut sind nicht so hoch und nicht so eng begrenzt als jene der Bienenbrut. Demgemäss hat der Wärmehaushalt beim Wespenvolk eine besondere Note, wenn auch bei einigen Arten viel Ähnlichkeit mit dem Verhalten des Bienenvolkes besteht.

Verfasser (1927, *b*) hat den Wärmeverlauf in einem Nest mittlerer Grösse von *Vespa vulgaris* in der Zeit von Ende Juli bis Anfang Oktober verfolgt und in der gleichen Zeit die Wärmeverhältnisse eines brütenden Bienenvolkes gemessen. Die abgebildeten Temperaturkurven (Abb. 6) stellen die Bruttemperatur des Wespennestes und des Bienenstockes dar. Während das Bienenvolk die Schwankungen der Aussentemperatur in vollendeter Weise zu kompensieren vermag, zeigt die Wärmekurve des Wespennestes kleine und zwar gleichsinnige Schwankungen, die verzögert eintreffen. Höchste und tiefste Nesttemperatur liegen im Wespennest um 10°, im Bienennest nur um 2·8° C auseinander. Die mittlere Schwankung beträgt 2·51° bei den Wespen und nur 0·55° bei den Bienen. Die Durchschnitte der täglichen Minima ergeben im Wespennest einen Wert von 29·46° C, die täglichen Maxima von 31·97° C. Dieser Temperaturbereich dürfte dem Optimum der Brutentwicklung für *Vespa vulgaris* entsprechen. Von Mitte September an nimmt die

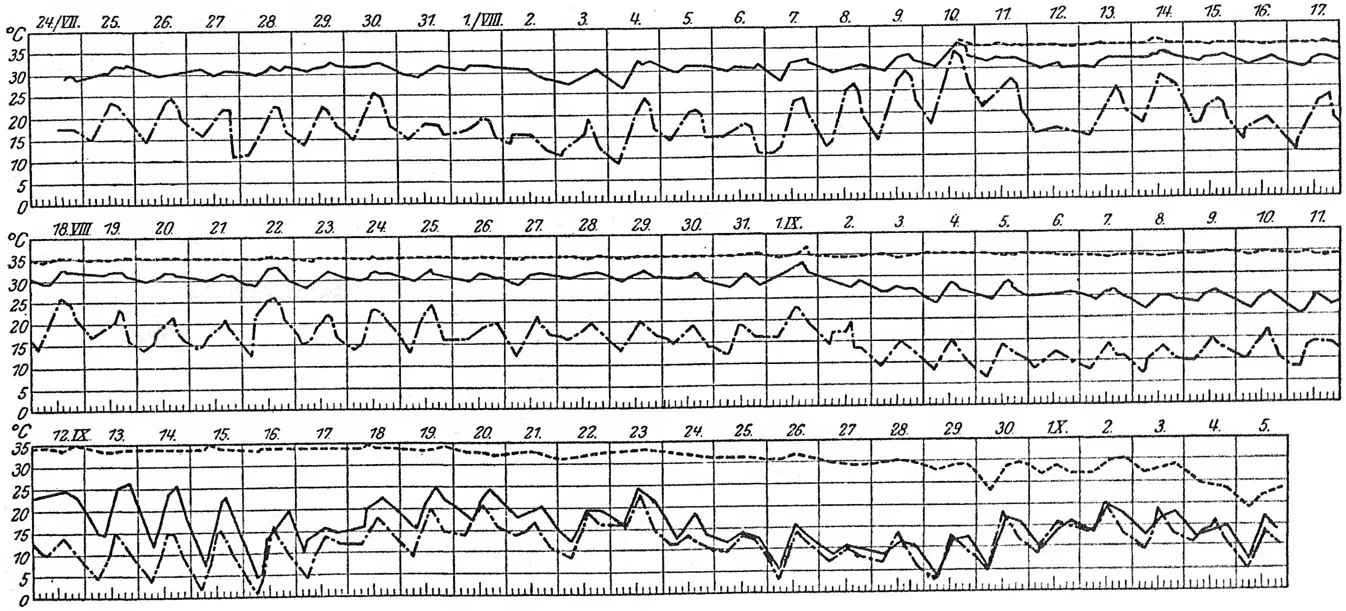

Abb. 6. Temperaturkurven eines Wespennestes und eines Bienenstockes in der Zeit vom 24. Juli bis 5. Oktober. (Aus Himmer.) ———— Temperatur im Wespennest, ········· Temperatur im Bienenstock, ·–·–·–·–·– Aussentemperatur.

Volksstärke im Wespennest mehr und mehr ab, was sich in einem auffallenden Rückgang der Nesttemperatur verbunden mit starken Schwankungen kundgibt. Aber auch das inzwischen brutlos gewordene Bienenvolk hält seine Bruttemperatur nicht mehr, die Nestwärme schwankt im Sinne der Aussentemperatur, solange diese nicht unter die Grenze von 7° C sinkt.

Einen ganz ähnlichen Wärmehaushalt wie *Vespa vulgaris* hat unsere grösste soziale Wespenart *Vespa crabro* (Abb. 7). Hier betrug nach Messungen des Verfassers (1931) in der Zeit von Ende Juli bis Mitte September der Abstand zwischen höchster und tiefster Nesttemperatur 6·9° C, der mittlere Schwankungswert 1·97° C. Der dem Brutentwicklungsoptimum entsprechende Temperaturbereich liegt zwischen 29·8 und 31·77° C. Nachstehende vergleichende Übersicht nach Himmer gibt ein aufschlussreiches Bild der Wärmeverhältnisse bei Bienen und Faltenwespen mit geschütztem Nestbau:

Temperaturwerte	Bienenstock (° C)	Wespennest (° C)	Hornissennest (° C)
Höchst gemessene Temperatur	36·0	36·0	33·1
Niederste Temperatur	33·2	26·2	26·0
Abstand zwischen höchster und tiefster Temperatur	2·8	10·0	6·9
Mittlere Temperatur	34·8	30·71	30·78
Mittelwert der täglichen Maxima	35·08	31·97	31·77
Mittelwert der täglichen Minima	34·53	29·46	29·80
Mittlerer Schwankungswert	0·55	2·51	1·97
Geringster Abstand von der Aussentemperatur	2·0	2·0	2·0
Grösster Abstand von der Aussentemperatur...	32·5	20·0	26·5
Durchschnittlicher Abstand von der Aussentemperatur	16·36	12·27	15·38

Die Mittelwerte der Nesttemperaturen der Hornissen und gemeinen Wespen sind nahezu gleich, während die Durchschnittstemperatur im Brutnest der Bienen um 4° höher liegt. Aber auch sonst ist ein deutlicher Unterschied zwischen Bienen und Wespen bei jenen Zahlen zu erkennen, die die Regulationsfähigkeit belegen. Abstand zwischen niederster und höchster Temperatur sowie mittlerer Schwankungswert sind bei den Bienen viel geringer als bei den Wespen, mit anderen Worten: die Bienen vermögen weit vollkommener zu regulieren als die Wespen. Unter den beiden aufgeführten Wespenarten besitzen zweifellos die Hornissen das bessere Wärmeregulationsvermögen.

Inwieweit die Wespen in der Lage sind, die Temperatur auch nach oben abzustoppen, also physikalisch zu regulieren, zeigt ein Versuch, den Verfasser (1931) nach dem Vorbilde von Hess durchgeführt hat. Da das Versuchsvolk in einem geschlossenen Kasten untergebracht war, konnte mit Hilfe eines elektrischen Heizgitters die Umgebungstemperatur beliebig erhöht werden. Erst bei einer Temperatur von 37° C im Brutnest begannen die Hornissen zu fächeln mit dem Erfolg, dass die Nesttemperatur etwas abgebremst wurde und gegen die Aussentemperatur zurückblieb. Das dauerte aber nicht lange, die Nesttemperatur stieg wieder weiter, weil die Hornissen nunmehr in Massen aus dem Nest flüchteten und die Brut im Stiche liessen. Die Nesttemperatur stieg bis 39·2° bei 40·7° Aussentemperatur, also

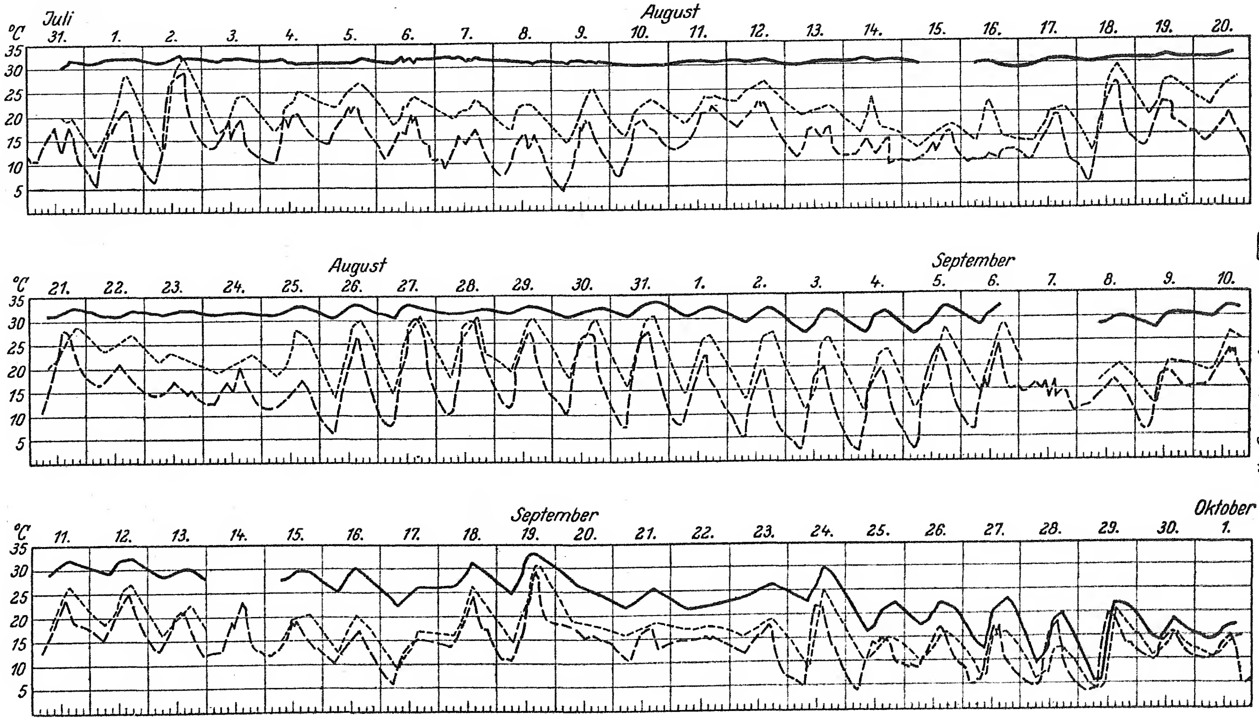

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Abb. 7. Temperaturkurve eines Hornissennestes in der Zeit vom 31. Juli bis 1. Oktober. (Das Nest war in einem geschlossenen Kasten untergebracht.) (Aus Himmer.)
———— Nesttemperatur, ·········· Kastentemperatur, - - - - - - - Aussentemperatur.
]

weit über den Optimalbereich hinaus. Die Abwehrreaktion hatte somit nur geringen Erfolg. Eine andere Kühlreaktion als das Fächeln konnte nicht beobachtet werden; Wasser wurde nicht eingetragen, obwohl Wasserstellen in unmittelbarer Nähe vorhanden waren. Aus diesem Verhalten ist zu schliessen, dass das physikalische Regulationsvermögen bei den Hornissen mangelhaft entwickelt ist. Das gleiche dürfte bei *Vespa vulgaris*, deren Lebensweise und Nestbauart mit dem grösseren Gattungsverwandten übereinstimmt, der Fall sein.

Wesentlich anders dagegen liegen die Verhältnisse bei den kleinen Feldwespen (*Polistes*), deren Nest ohne schützende Hülle den Einflüssen der Umgebung preisgegeben ist. Um die Sonnenwärme auszunützen bauen die Feldwespen ihre Nester gewöhnlich an geschützter, nach Süden gerichteter Stelle. Wie oben mitgeteilt, ist bei den Feldwespen die Fähigkeit der Erzeugung von Körperwärme wenig ausgebildet. Sie können daher ihre Brut kaum vor Abkühlung schützen und es scheint auch, dass diese gegen tiefere Temperaturen wenig empfindlich ist. Andererseits aber besteht durch die direkte Sonnenbestrahlung, der das Nest ausgesetzt ist, die Gefahr der Überhitzung. Gegen diese Gefahr wissen sich die Feldwespen (*Polistes gallicus* var. *biglumis*), wie Steiner (1930) in vorbildlichen Versuchsreihen nachgewiesen hat, in wirksamer Weise zu schützen (Abb. 8). Weibchen und Hilfsweibchen regulieren zunächst die Temperatur durch anhaltendes, kräftiges

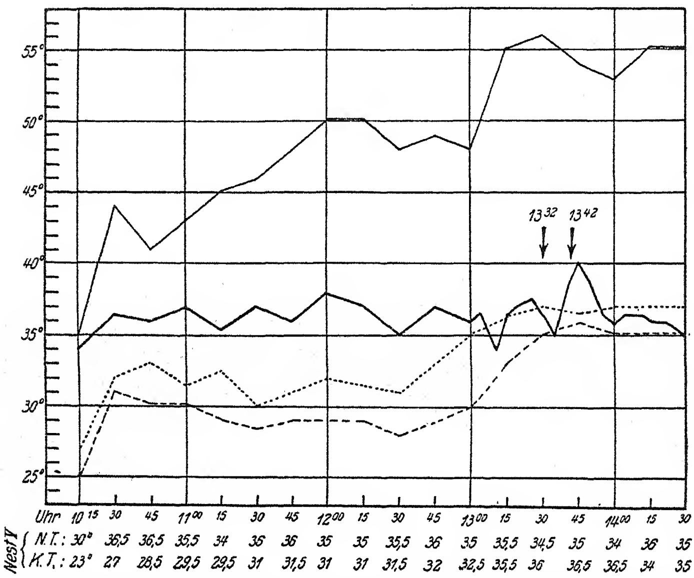

Abb. 8. Temperatur-Zeitkurven des Versuchsnestes III und des leeren Kontrollnestes II am 3. August 1929. (Nestreihe besonnt, Kontrollreihe beschattet. Ablesungen: Bei Nest III bis 13 Uhr 15-minütlich, nachher 5-minütlich. Bei Nest II 15-minütlich.)

Versuchsnest III	*Leeres Kontrollnest II*
———— Nesttherm.	———— Nesttherm.
············ Kontrolltherm.	– – – – – – – Kontrolltherm.

13.32–13.42 Einsperrung der Königin während 10 Minuten. 1, Abflug zum Wassertransport um 10.52. *N.T.* = Nesttemperatur; *K.T.* = Kontrolltemp. von Nest V.

Fächeln. Wenn diese Massnahme nicht ausreicht, dann schleppt das Weibchen (Königin) Wasser herbei, das sie in feinen Tropfen in die Zellen verteilt. Dann wird das Fächeln fortgesetzt, das eingetragene Wasser verdunstet und die auftretende Verdunstungskälte schafft den notwendigen Ausgleich. Trotz starker Überhitzung der Umgebung halten die Wespen im Brutnest die Optimaltemperatur von 35·5° C, bei der sich die Brut am besten entwickelt, mit Erfolg fest. Steiner fand z.B. bei einem besetzten *Polistes*nest während sechs Sonnentage eine mittlere Nesttemperatur von 36·43° C; die Durchschnittstemperatur eines leeren Kontrollnestes, das den gleichen Umgebungseinflüssen ausgesetzt war, betrug dagegen 47·7° C. Die Wespen hatten also mehr als 12° abgestoppt. Der Versuch erweist zugleich die hohe Wärmeabsorptionsfähigkeit des Nestmaterials. Der Wassertransport ist an den Optimal- und Überoptimaltemperaturbereich gebunden. Fällt die Nesttemperatur unter das Optimum, so erfolgt zwischen 35 und 31° eine Umkehr des bisherigen Verhaltens. Die Wespen saugen das eingetragene Wasser wieder ab und speien es aus, um ein unnötiges Abkühlen zu vermeiden. Eine Königin kann in einer Stunde 3 ccm. Wasser eintragen, das einer Verdampfungswärme von 1830 Kal. entspricht. Wenn die Aussentemperatur sinkt, dann kühlt das Nest aus, ohne dass die Wespen in der Lage sind, auch nur vorübergehend eine höhere Temperatur zu erzeugen. Die Brut ist einzig und allein auf die Umgebungswärme, vor allem auf die Insolation angewiesen. Die Feldwespen sind also nicht zur chemischen Wärmeregulation befähigt, sie würde auch gar keinen Zweck haben, da das hüllenlose, aus einer einzigen Wabe bestehende Nest eine Wärmespeicherung unmöglich macht.

Sehr lehrreich ist ein Vergleich der Wärmeleistung des Körpers mit der optimalen Brutnesttemperatur einiger sozial lebenden Hautflügler. Die nachstehenden Zahlen stellen Mittelwerte aus einer Reihe von Messungen dar:

Art	Mittlerer Wärmeüberschuss im Körper gegenüber der Umgebung °C	Brutnesttemperatur ° C		
		Im Mittel	Mittlerer Schwankungswert	Durchschnittlicher Abstand von der Aussentemperatur
Apis mellifica	12·40	34·8	0·55	16·36
Vespa vulgaris	10·48	30·71	2·51	12·27
Polistes gallicus	3·08	Nahe der Umgebungstemperatur, grösserer Abstand nur bei Überhitzung		

(3) DER WÄRMEHAUSHALT DER AMEISEN.

Bisher handelte es sich um den Wärmehaushalt von wabenbauenden Hautflüglern, deren Brut und Vorräte örtlich streng gebunden sind. Die Jungtiere verbleiben vom Augenblick der Eiablage während der ganzen Entwicklung bis zum Schlüpfen als Imago in ein und derselben Zelle. Der Wärmebezirk im Bienenstock und im Wespennest ist somit topographisch durch die Brut bestimmt. Die Brut kann nicht in beliebige Nestteile verlegt werden, die etwa durch äussere Einflüsse thermisch begünstigt sind. Aufgabe der Imagines ist es, jene Nestteile zu erwärmen, die die Königin zur Ablage ihrer Eier auserwählt hat. Wahrscheinlich wird für die

Eiablage durch die Königin das Vorhandensein einer dem Entwicklungsoptimum nahen Temperatur massgebend sein.

Ganz andere Voraussetzungen finden sich in dieser Beziehung im Ameisennest und bedingen dementsprechend einen anderen Typus des Wärmehaushaltes.

Die Ameisenbrut ist nicht lokal gebunden. Sie kann nach Belieben umgetragen werden. Es besteht daher die Möglichkeit, die durch äussere Wärmeeinflüsse bedingten Wärmezustände entsprechend auszunützen. Schon für den Wärmehaushalt der Feldwespen spielen die äusseren Einflüsse eine erhebliche Rolle. Diese Einstellung auf physikalische Umgebungszustände ersetzt beim Ameisenstaat fast gänzlich die chemische Wärmeregulation.

Das Nest der Waldameise (*Formica rufa*) besteht aus einem oberirdischen und einem unterirdischen Teil. Der unterirdische Teil ist in das gewachsene Erdreich gegraben, der oberirdische Teil, die Kuppel, ist künstlich aus Pflanzenmaterial und Erde aufgeführt. Die Kuppel enthält im Innern Hohlräume und ist nach aussen von einer dichter gefügten Decke abgeschlossen. Ins Freie führen verschiedene Öffnungen, die nach Belieben geöffnet oder geschlossen werden können. Über den Wärmehaushalt der Waldameise (*Formica rufa* var. *rufo-pratensis*) hat Steiner (1924, 1925) sehr eingehende Untersuchungen veröffentlicht.

Die optimale Bruttemperatur der Waldameisen liegt zwischen 23 und 29° C und wird in einer Nesttiefe von 15–50 cm dauernd erhalten. Die Bodentemperatur liegt ungefähr 10° tiefer als das Mittel der Nesttemperatur. Die physikalischen Faktoren für den Wärmehaushalt der Ameisen sind Boden- und Lufttemperatur sowie Wind als abkühlende Komponenten, die Insolation als Wärmespender. Begünstigt wird der Wärmeauffang durch südliche, windstille Lage des Nestes, sowie durch entsprechende von Einstrahlungs- und Niederschlagsverhältnissen bestimmte Formgebung. Bei tiefem Sonnenstand vergrössert sich die Auffangfläche für die Wärmestrahlen. Die durch Insolation erzielte Wärme unterliegt einer weiteren Regelung derart, dass nach Bedürfnis die Nesteingänge auf der Schatten- oder Sonnenseite geöffnet bezw. geschlossen werden, und dass ferner die Brut näher oder entfernter der Kuppeloberfläche untergebracht wird. Bei kühlen Umgebungstemperaturen, also z.B. nachts, ziehen sich die Waldameisen mit ihrer Brut in die Nesttiefe zurück, drängen sich enger zusammen und können unter Umständen eine geringe Eigenwärme erzeugen. Neben der Funktion als Wärmeauffang gewinnt die Nestkuppel auch als Wärmespeicher eine gewisse Bedeutung. Die Wärmespeicherung ist naturgemäss umso grösser und dauerhafter je umfangreicher das Nest angelegt ist. Speichervermögen des Nestes und physiologische Wärmeerzeugung der Ameisen bewirken, dass auch die tieferen Nestteile erheblich wärmer als der umgebende Boden sind und längere Zeit Bruttemperatur zu halten vermögen. Die Optimaltemperatur wird bei den Ameisen nicht so genau eingehalten wie bei den Bienen, besonders nicht in jüngeren, kleineren oder auch altersschwachen Kolonien, sowie bei ungünstiger Durchschnittswitterung. Im Herbst sinkt die Nesttemperatur bedeutend ab unter dem Einfluss der zunehmenden Kälte und infolge Nahrungsmangels. Die Ameisen ziehen sich dann von der Kuppel in den unterirdischen Nestteil zurück, der nunmehr besser temperiert ist, als die immer seltener von der

Sonne beschienene Kuppel. Während des Winters haben die Ameisen keine nennenswerte Eigenwärme. Ihre Körpertemperatur stimmt ungefähr mit der Nesttemperatur und diese mit der Bodentemperatur überein. Die Ameisen suchen im Winter Bodenschichten auf, deren Temperatur gerade noch über dem Gefrierpunkt liegt. Sie selbst leben in einem Zustande der Kältestarre. Die Wärmeregulation der Waldameisen hat also nur während der Sommerzeit einen aktiven Charakter, im Winter erfordert die gleichmässige Bodenwärme keine Abwehrreaktion, zumal ja auch keine Brut vorhanden ist.

Noch einmal hervorgehoben sei, dass die Waldameisen die Fähigkeit der chemischen Wärmeerzeugung, wenn auch in geringem Masse, besitzen und dass diese Fähigkeit die soziale Wärmeregulierung unterstützt. Sie sind darin den Feldwespen überlegen, denen eine chemische Wärmeregulation vollständig zu fehlen scheint. Man darf freilich nicht übersehen, dass eine solche im *Polistes*nest wegen seiner offenen Bauart und der geringen Insassenzahl gar nicht möglich ist.

Auf einer tieferen Stufe steht der Wärmehaushalt bei Ameisenarten, die entweder rein minierte Erdnester oder Erdnester mit kleiner Kuppel aufführen. Die Erdkuppelnester von *Lasius niger*, *Lasius flavus*, *Formica fusca* und das kombinierte Nest von *Formica exsecta* verfügen nach Steiner (1929) zwar über einen bedeutenden Wärmeauffang, die Wärmespeicherung dagegen ist in diesen Nestern nicht ausreichend, die Wärme dauernd über der Bodentemperatur zu halten und so z.B. die nächtliche Abkühlung zu vermeiden. Nur das kombinierte Nest von *Formica exsecta* zeigt Anfänge einer Dauerwirkung und bildet somit den Übergang zum Waldameisennest (Abb. 9). Das Wesentliche bei diesen Nestern ist der Wärmeauffang, der sich der nächsten Umgebung mitteilt. Besonders bei niederen Sonnenständen ist die Insolation ein vorzügliches Mittel zur raschen Erwärmung des Nestes, die von den Ameisen durch entsprechende Brutverschiebung nutzbar gemacht wird. Der Grad der Wärmespeicherung richtet sich nach der Grösse der Kuppel, nach der Form des Nestes, nach dem Baumaterial und schliesslich nach der Bewachsung der Kuppeloberfläche. Die Wärmekapazität ist kleiner als beim umgebenden Erdboden, die Kuppel ist somit grösseren Schwankungen unterworfen als der Boden.

Bei minierten Nestern, die unter Steinen angelegt werden (*Lasius niger*, *L. flavus*, *Formica fusca*, mehrere *Myrmica*-Arten und *Tetramorium caespitum*), fällt auch die Wärmespeicherung weg. Der Wärmeverlauf wird durch die physikalischen Eigenschaften des Steines bestimmt. Einer raschen Erwärmbarkeit steht eine ebenso rasche Abkühlung gegenüber (Abb. 10). Steiner kennzeichnet den Stein als "eine in das Insolationsfeld hinausragende Wärmeantenne." Besonders in rauhen Gebirgslagen gewinnen die Steinnester eine erhöhte Bedeutung, da die notwendigen Bruttemperaturen nur noch in der Umgebung von Steinen erzielt werden können. Da andererseits die Steinnester starken Temperaturschwankungen ausgesetzt sind, ist ein häufiger Bruttransport erforderlich. Während der Nachtzeit wird die Brut in den tieferen Nestpartien, am Morgen und Vormittag in den obersten Schichten in der Nähe des Steines, über Mittag bis in den Nachmittag hinein in den mittleren Schichten und gegen Abend wieder in der Nähe der Oberfläche gehalten.

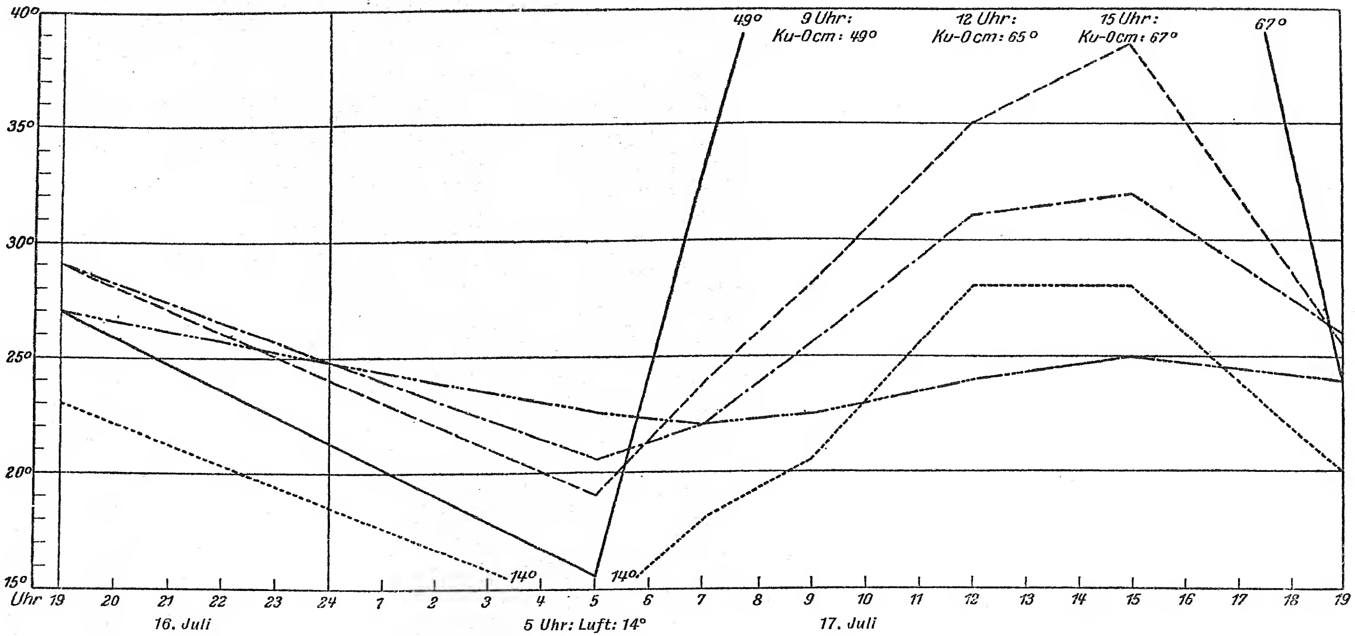

Abb. 9. Temperatur-Zeitkurven der Kuppel von *F. exsecta VI*, 16.–17. Juli, 1928. (Aus Steiner.)

——— Tiefe 0 cm. – – – – – – Tiefe 5 cm. –.–.–.– Tiefe 10 cm.
–...–...– Tiefe 20 cm. .... Lufttemperatur.

In all diesen Nestern konnte Steiner keinerlei Anzeichen einer chemischen Wärmeregulation feststellen. Die Wärme liefernden Faktoren sind lediglich physikalischer Natur.

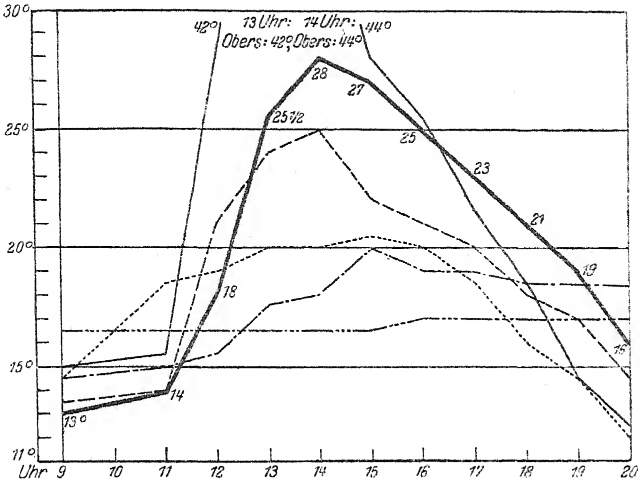

Abb. 10. Temperatur-Zeitkurven und Bruttransport der Stein-Nestanlage von *L. flavus VIII*, 6. August, 1928. (Aus Steiner.)

——— Steinoberseite. ——— Steinunterseite (= Nestanlage). ------ Bodenoberfläche.
-·-·-·- Boden, Tiefe 10 cm. -···-···- Boden, Tiefe 20 cm. ········ Lufttemperatur.

Bruttransport: 9 Uhr, 13°: Keine Brut, nur einige Arbeiter. 11 Uhr, 14°: Befund wie um 9 Uhr. 12 Uhr, 18°: Keine Brut, aber grosse Menge von Arbeitern. 13 Uhr, $25\frac{1}{2}$°: Brut (Puppen) im hinteren Teil der Nestanlage ausgelegt; Stärke 2; in der Tiefe der Gänge Zutransport sichtbar. 14 Uhr, 28°: Nur noch 1 Puppe vorhanden: in den Gängen Abtransport feststellbar. 15 Uhr, 27°; Grosse Menge von Puppen, Brutkammern gefüllt; Stärke 3. 16 Uhr, 25°: Wie um 15 Uhr, Stärke 3. $16\frac{1}{2}$ Uhr, 24°: Wie vorher, Stärke 3. 17 Uhr, 23°: Kleinere Menge Puppen, Stärke 2. $17\frac{1}{2}$ Uhr, 22°: Wie um 17 Uhr, Stärke 2. 18 Uhr, 21°: Kleine Menge Puppen, Stärke 1. $18\frac{1}{2}$ Uhr, 20°: 2 Puppen; Abtransport. 19 Uhr, 19°: Keine Brut, nur Arbeiter.

IV. ÜBERSICHTLICHE DARSTELLUNG DES WÄRMEHAUSHALTES VON SOZIALEN HYMENOPTEREN.

Die angeführten Arbeiten haben unsere Kenntnisse von den Wärmeverhältnissen sozialer Insekten wesentlich erweitert. Das zu erforschende Gebiet ist aber noch lange nicht erschöpft. Die durchgeführten Untersuchungen beziehen sich auf Länder der gemässigten Zone. Unter anderen klimatischen Bedingungen dürfte das thermische Verhalten der erwähnten Arten ein entsprechend anderes sein.

Man könnte den Versuch machen, die bisher festgestellten Tatsachen nach bestimmten Gesichtspunkten zu ordnen, um eine übersichtliche Darstellung der Zusammenhänge von Lebensweise und Wärmehaushalt zu gewinnen. Nachstehende Zusammenfassung ist unter teilweiser Benützung einer von Steiner (1931) verfassten Übersicht aufgestellt worden. Sie macht nicht den Anspruch einer endgiltigen Einordnung unserer bisherigen Kenntnisse, sondern sie soll lediglich richtungweisend für weitere Fragestellungen sein.

Art	Nestbau	Temperaturoptimum der Brutentwicklung	Wärmehaushalt	
			Chemische	Physikalische
			Regulation	
Apis mellifica (Volksstärke ca. 20,000–70,000 Einzeltiere)	Wabenbau aus Wachs in geschlossenem Raum, Vorratsanlage, gute Wärmespeicherung	34–35° C, wird streng eingehalten	Muskelwärme, Temp.-überschuss i.M. 12·4° C	Wasserdampfabgabe beim Atmen, Ventilation durch Fächeln, Wassertransport, Vorrats u. Brutmassen als Wärmepuffer, Traubenbildung im Winter
Vespa vulgaris u. *Vespa crabro* (ca. 100–1000 Einzeltiere)	Wabenbau mit Hülle aus Holzfaserstoff in geschlossenem Raum, keine Vorratsanlage, geringe Wärmespeicherung	29½–32° C, wird nicht genau eingehalten	Muskelwärme, Temp.-überschuss i.M. 10·5° C	Wasserdampfabgabe beim Atmen, Ventilation durch Fächeln, Brutmasse als Wärmepuffer
Polistes gallica (20–50 Einzeltiere)	Wabenbau ohne Hülle aus Holzfaserstoff, einwabig im Freien, in sonniger Lage, keine Wärmespeicherung, hohes Wärmeabsorptionsvermögen	35·4° C, wird nur bei Sonnenbestrahlung eingehalten, sonst Umgebungstemperatur	Keine chemische Wärmeregulation, Temp.-überschuss i.M. 3° C	Insolation, Ventilation durch Fächeln, Wassertransport
Formica rufa (10,000–100,000 Einzeltiere)	Kombiniertes Nest, Kuppelbau aus Erde und pflanzlichen Stoffen, innere Hohlräume, feste, geschlossene Kuppeldecke, gute Wärmespeicherung	23–29° C, wird im Sommer annähernd eingehalten	Geringe Muskelwärme	Wärmeauffang durch Nestkuppel, Wärmespeicherung, Öffnen u. Schliessen der Nestöffnungen, Brutverschiebung, Haufenbildung
Formica exsecta	Kombiniertes Nest, dünne lockere Kuppeldecke, kleine Innenräume, Wärmespeicherung	23–29° C	Fraglich	Wärmeauffang durch Nestkuppel, Wärmespeicherung, Brutverschiebung
Ameisen in grösseren, dauerhaften Erdkuppeln (*Lasius niger*, *L. flavus*) 5000–10,000 Einzeltiere)	Miniertes Nest mit Nestkuppel über 15 cm hoch, geringe Wärmespeicherung	—	Keine chemische Wärmeregulation	Wärmeauffang, geringe Wärmespeicherung, Brutverschiebung
Ameisen in kleinen vergänglichen Erdkuppeln und unter Steinen (*Formica fusca* u. *Tetramorium caespitum*, *Myrmica*-Arten)	Miniertes Erdnest mit Kuppel unter 15 cm Höhe oder unter Steinen	—	Keine chemische Regulation	Wärmeauffang und Wärmeübertragung ohne nennenswerte Wärmespeicherung, häufige Brutverschiebung
Ameisen in rein minierten Nestern	Miniertes Nest ohne Kuppel und ohne Stein	—	Keine chemische Regulation	Nesttemperatur gleich Bodentemperatur, Brutverschiebung im Boden

V. ZUSAMMENFASSUNG.

In den Nestbauten staatenbildender Hautflügler spielt die Wärme eine wichtige biologische Rolle. Die *Körpertemperatur* vieler Insekten, insbesondere der sozialen Hymenopteren, kann unter bestimmten Verhältnissen die Temperatur der Umgebung vorübergehend beträchtlich überschreiten. Das Höchstmass der Wärmeentfaltung ist arttypisch verschieden. Aber auch innerhalb der Art zeigen sich Verschiedenheiten je nach Lebensalter und Geschlecht. Bei *Apis mellifica* ergaben grössere Messreihen folgende mittlere *Temperaturüberschüsse*: ♂ 13·73° C, ♀ 5·72° C, ☿ 12·4° C. Bei *Vespa vulgaris* sind die Temperaturüberschüsse mit Ausnahme der ♀ geringer, nämlich: ♂ 6·24° C, ♀ 10·48° C und ☿ 7·46° C. Die geringste Wärmeentfaltung wurde bei *Polistes*-Arten gefunden. Hier betrug der Temperaturüberschuss im Mittel für die drei Formen nur 3·08° C. Körpertemperaturmessungen an Ameisen fehlen bisher, doch dürfte ihre Körpertemperatur nur wenig verschieden von der Aussentemperatur sein.

Soweit die Nestwärme in der Hauptsache durch Körperwärmeabgabe seitens der Nestinsassen aufgebracht wird, ist eine deutliche Beziehung zwischen Wärmeentfaltung und Brutentwicklungsoptimum ersichtlich. Das *Entwicklungsoptimum* der Honigbiene liegt bei 35° C, von *Vespa vulgaris* zwischen 29·5° und 32° C und von *V. crabro* zwischen 29·8° und 31·8° C. Bei Arten, deren Nester geringe Wärmespeicherung haben und deshalb starken Temperaturschwankungen ausgesetzt sind, ist das Temperaturoptimum der Brutentwicklung sehr unscharf fixiert. Während bei der Honigbienenbrut die Optimal- und Vitalgrenzen sehr nahe beisammen liegen, vertragen Wespen, Hummeln und Ameisen erhebliche Abweichungen von den Optimalgrenzen, besonders nach unten. Bei Ameisen sind folgende Entwicklungsoptima festgestellt: *Formica rufa*, 23–29° C, *F. fusca*, obere Grenze, bei 35–36° C, *Lasius flavus*, 23–28° C, *L. niger*, obere Grenze, 31° C, *Myrmica rubra-ruginodis*, obere Grenze, 31° C, *Tetramorium caespitum*, obere Grenze, 31° C. Obwohl der Wärmeüberschuss der Feldwespen ein geringer ist, liegt das Entwicklungsoptimum sehr hoch, bei 35·5° C.

Die untere *Aktivitätsgrenze* (Kältestarre) der Honigbiene liegt bei etwa 7° C, bei Wespen, Hummeln und Ameisen nahe bei 0°C.

Allen sozialen Hymenopteren ist ein gut ausgebildeter *Temperatursinn* zu eigen. Bei *Formica rufa* wurde eine Empfindlichkeit für Temperaturunterschiede von 0·5° C. (nach Herter) nachgewiesen.

Der *Wärmehaushalt* der sozialen Hymenopteren weist verschiedene Typen auf je nach der Bauart des Nestes und der Fähigkeit eigener Wärmeentfaltung. Am besten ist der *Wärmehaushalt bei der Honigbiene* ausgebildet.

Im Sommer herrscht im Brutbezirk des Bienenstockes eine konstant bleibende Temperatur von 35° C mit durchschnittlichen Tagesschwankungen von 0·5° C. Auch bei Überhitzung vermögen die Bienen durch Fächeln und vermutlich auch durch Wassertransport so abzustoppen, dass die obere Vitalgrenze (36° C) nicht überschritten wird. Die Honigbienen besitzen sowohl die Fähigkeit der chemischen als auch der physikalischen Wärmeregulation. Brutmasse, Vorräte (Honig und Pollen), sowie Wabenbau dienen dank ihrer hohen Wärmekapazität als Wärmepuffer.

Während der brutfreien Winterzeit ziehen sich die Bienen zwischen den Waben zu einer dichten Traube zusammen. Die Bienentraube hat einen Wärmeinhalt, der sich aus der Summe der geringen Wärmemengen ergibt, die von den einzelnen Bienenkörpern abgegeben werden. Von der Mitte der Bienentraube fällt die Wärme allmählich gegen den Rand zu ab. Die Randbienen sind den Ausseneinflüssen, also der Gefahr der Erstarrung am meisten ausgesetzt. Die Temperatur am Traubenrand darf nicht unter 9° C (nach Hess 8° C) herabsinken. Im Traubeninnern sind die Bienen mehr aktiv und erzeugen nach Bedarf Wärme. Diese Wärmeerzeugung ist dahin gerichtet, am Traubenrand die lebensnotwendige Mindesttemperatur (motorisches Minimum) zu halten. Deshalb steigert sich die Wärme in der Bienentraube, wenn die Aussentemperatur sinkt und lässt nach, wenn diese steigt. Die Regulation wird durch engeren Zusammenschluss bezw. durch Lockerung der Bienentraube wirksam unterstützt. Erreicht die Umgebungstemperatur eine Höhe von über 8° C, dann verlaufen die Schwankungen der Traubentemperatur im Sinne der Aussentemperatur, d.h. die Wärmeregulation wird überflüssig, solange noch kein Brutnest vorhanden ist.

Ähnlich wie die Honigbiene regulieren die *Wespen* mit geschlossenem und geschütztem Nestbau (kalyptodome Nester). Der Wärmeverlauf im brütenden Wespenvolk (*Vespa vulgaris*, *V. crabro*) ist jedoch weniger gleichmässig, es treten geringe Schwankungen im Sinne der Aussentemperatur auf. Während bei den Wespen die chemische Wärmeregulation noch verhältnismässig gut ausgebildet ist, ist die physikalische Regulation mangelhaft, sie beschränkt sich auf Ventilation und Wasserdampfabgabe durch die Brut. Die mehrschichtige Nesthülle mit eingeschlossenen Lufträumen unterstützt die Wärmeregulation der Wespen in wirksamer Weise.

Die *Feldwespen* (*Polistes*) mit ihren ungeschützten, hüllenlosen (gymnodomen) Nestern verzichten vollständig auf die chemische Wärmeregulation. Die notwendige Brutwärme wird durch Wärmeauffang erzielt. Sehr ausgeprägt dagegen ist die physikalische Stoppreaktion, die bei drohender Überwärmung (direkte Sonnenbestrahlung) prompt einsetzt. Sie beginnt mit ausgiebigem Fächeln, um schliesslich in Verbindung mit Eintragen von Kühlwasser in die Zellen ihren höchsten Wirkungsgrad zu erreichen. Da Wärmespeicherung und chemische Wärmeregulation fehlen, kühlt das Nest bei Sonnenuntergang bis auf Umgebungstemperatur aus.

Der Wärmehaushalt der Ameisenstaaten ist durch weitgehende Abhängigkeit von den Ausseneinflüssen gekennzeichnet. Je nach Grösse und Bauart der Nestkuppeln ist die Wärmespeicherung mehr oder weniger ergiebig und dauerhaft. Der Kuppelbau gestattet in allen Fällen einen bedeutenden Wärmeauffang. In den kombinierten Nestern der *Waldameisen* (*Formica rufa*) ist unter Mitwirkung der chemischen Wärmeproduktion eine Dauerwirkung in ungefährer Höhe der optimalen Bruttemperatur erreicht. Auch im Nest von *Formica exsecta* hält die aufgespeicherte Wärme längere Zeit an, sie bleibt jedoch nicht konstant, sondern sinkt während der Nacht unter den Optimalbereich. Chemische Wärmeerzeugung konnte nicht nachgewiesen werden. Die einfachen *Erdkuppelnester* (*Lasius niger*, *L. flavus*, *Formica fusca*) haben eine zu geringe Wärmespeicherung, als dass bei Aussetzen der Insolation eine Abkühlung auf Bodentemperaturhöhe und noch niedriger zu verhindern wäre. Chemische Wärmeerzeugung kommt hier nicht in Frage. Eine aktive Wärmeregulierung erfolgt wie bei den Waldameisen durch Verschliessen bezw. Öffnen der Nestausgänge sowie Transport der Brut in optimale Wärmeschichten. Bei *Ameisennestern*, die unter Steinen angelegt werden (*Lasius niger*, *Formica fusca*, verschiedene *Myrmica*-Arten, *Tetramorium caespitum*), fällt dem Stein die Rolle als Wärmevermittler zu. Vermöge seiner guten Wärmeleitungsfähigkeit wird das Nest rasch temperiert, aber auch ebenso rasch wieder abgekühlt. Die Steinnester sind daher starken Temperaturschwankungen unterworfen. Die flüchtigen Wärmeperioden werden durch häufigen Bruttransport ausgenützt.

Im Winter ziehen sich die Ameisen in tiefere Schichten des Bodennestes zurück, wo die Temperatur knapp über dem Gefrierpunkt verharrt. Sie befinden sich während der kalten Jahreszeit in einem der Kältestarre nahen Zustand ohne jegliche Eigentemperatur.

VI. SUMMARY.

Heat plays an important biological rôle in the nests of social insects. Under particular circumstances the *body temperature* of many insects, especially social Hymenoptera, may temporarily exceed the temperature of the surroundings by a measurable amount. The extent of heat production varies with species, and within a given species there are differences depending on age and sex. In the case of *Apis mellifica* the following mean temperature excess was found as a result of numerous measurements: ♂ 13·73° C., ♀ 5·72° C., ☿ 12·4° C. In *Vespa vulgaris*, with the exception of females, the temperature excess was less, namely: ♂ 6·24° C., ♀ 10·48° C., ☿ 7·46° C. The smallest heat production was found in *Polistes* species. Here the mean temperature excess for the three sex forms was only 3·08° C. No measurements of body temperature have been made up to the present with ants, but their body temperature is probably little above that of the environment.

When the temperature of a nest is raised mainly by an output of heat from the inhabitants, a marked relationship is evident between heat production and the temperature optimum for the development of brood. The developmental optimum for the honey bee is 35° C., for *Vespa vulgaris* between 29·5° and 32° C., and for *V. crabro* between 29·8° and 31·8° C. In species whose nests show little heat accumulation and are therefore subject to considerable temperature fluctuations the temperature optimum for brood development is not sharply defined. Whereas in the case of the honey bee optimal and vital limits lie very close together, wasps, humble bees and ants can support considerable variations from the optimal limits, particularly on the side of lower temperatures. The following developmental optima have been established for ants: *Formica rufa*, 23–29° C.; *F. fusca*, upper limit, 35–36° C.; *Lasius flavus*, 23–28° C.; *L. niger*, upper limit, 31° C.; *Myrmica rubra-ruginodis*, upper limit, 31° C.; *Tetramorium caespitum*, upper limit, 31° C. For field wasps, although the heat excess over the surroundings is smaller, the optimum temperature for development is very high, namely 35·5° C.

The lower *limit of activity* (cold rigor) for the honey bee is at about 7° C.; for wasps, humble bees and ants, close to 0° C.

All social Hymenoptera possess a well-developed *temperature sense*. *Formica rufa* is sensitive to differences of 0·5° C. (Herter).

The nature of *temperature regulation* in social Hymenoptera varies with the type of con-

struction of the nest and with the capacity for heat production by the individuals. Temperature regulation is best developed in the case of the honey bee. During the summer a constant temperature of 35° C., with average daily fluctuations of 0·5° C., prevails in the regions of the hive containing the brood. Moreover, when overheated the bees are able to keep down the temperature by fanning, and probably also by water transport, so that the vital limit (36° C.) is not exceeded. Indeed, honey bees possess the capacity both of chemical and of physical heat regulation. Brood, stores (honey and pollen) and comb all act as heat buffers, thanks to their high specific heats.

During the winter months, when there is no brood, the bees assemble between the combs, forming a closely packed bunch. This mass of bees has a heat content made up by the sum of the small quantities of heat given off by the separate bodies of the bees. The heat decreases progressively from the middle to the borders of the mass of bees. The outermost bees are most exposed to outside influences, that is to the danger of rigor. The temperature on the outskirts of the bunch must not fall below 9° C. (8° C. according to Hess). In the inside of the bunch the bees are more active and produce heat as required. This heat production has the purpose of maintaining the minimum temperature necessary for life (minimum for movement) on the outskirts of the mass of bees. Therefore the heat inside the bunch increases if the outside temperature falls, and decreases if this rises. The regulation is assisted by closer or looser packing of the mass of bees. When the surrounding temperature rises above 8° C., the temperature in the bunch varies in the same sense as the external temperature. Heat regulation becomes superfluous, so long as no brood is present.

Wasps which have closed and protected (calyptodomous) nests regulate heat in the same ways as bees. Nevertheless the temperature in nests of *Vespa vulgaris* and *V. crabro* containing brood is less uniform than in the case of honey bees. Small fluctuations occur in the same sense as the external temperature. Whereas in wasps chemical heat regulation is comparatively well developed, physical regulation is imperfect and is confined to ventilation and evaporation from the brood. But the many-layered nest wall with its enclosed air spaces considerably aids the heat regulation of wasps.

Field wasps (*Polistes*) with their unprotected wall-less (gymnodomous) nests completely abandon chemical heat regulation. The necessary heat for the brood is obtained by heat absorption. But a physical protective reaction against overheating is very well developed. This sets in as soon as there is a danger of overheating by direct solar radiation. Beginning with fanning, it culminates in the transport of cool water into the cells. Since heat accumulation and chemical heat regulation are absent, the nest cools down to the surrounding temperature at sunset.

The temperature conditions of *ants' nests* are characterised by considerable dependence on outside influences. According to the size and nature of construction of the nests the heat accumulation is more or less efficient and lasting. The dome-like shape of the nests allows in all cases of a considerable heat intake. In the composite nests of the wood ants (*Formica rufa*) a continuous high temperature at about the level of the optimum for brood development is attained by chemical heat production. In the nest of *Formica exsecta*, too, the accumulated heat is maintained for a considerable time. Nevertheless it is not constant but sinks at night below the optimum temperature, and no chemical heat production could be detected. The simple earth-dome nests (*Lasius niger*, *L. flavus*, *Formica fusca*) have too small a heat accumulation to prevent cooling down to ground temperature or even lower on the suspension of insolation. There is no question here of chemical heat production. Active heat regulation is accomplished, as in the case of wood ants, by opening and closing the nest entrances and by transport of brood to optimal heat levels. In the case of ants' nests constructed under stones (*Lasius niger*, *Formica fusca*, various species of *Myrmica*, *Tetramorium caespitum*) the stone assumes the rôle of heat mediator. Thanks to the good heat conduction of the stone the nest is rapidly warmed up, but just as rapidly cooled down again. Stone nests are, therefore, subject to great temperature fluctuations. Temporary periods of warmth are utilised by frequent transport of brood.

In winter ants retire into the deeper layers of the nest in the earth, where the temperature remains just above freezing point. During the cold season they are in a state approaching cold rigor, and have no body heat.

ACKNOWLEDGMENTS.

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COLLOIDAL STRUCTURE AND ITS BIOLOGICAL SIGNIFICANCE[1]

BY D. JORDAN LLOYD.

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(With Seventeen Text-figures.)

THE thesis which it is wished to develop in the course of this article can be stated very briefly—it is that all biological activities take place in the presence of two substances, namely protein and water, and unless each of these is present in minimal concentration the chemical reactions characteristic of living matter cannot occur. Moreover, it is suggested that the geometrical structure of the protein molecules is a factor of the greatest importance in controlling the ratio of water and protein present in any tissue. There is a ratio between water and protein at which biological activity is at a maximum and the decrease in both chemical and physical activity that is found in different tissues in conjunction with a decrease in the proportion of water present is shown to be a necessary consequence of the reorganisation of the colloidal structure that has occurred, the reorganisation taking the form of a simplification of structure and closer packing of the protein molecules, leading to an elimination of water—a process that culminates in protein structures such as the fibres of the connective tissue or hairs, in which all biochemical activity has been brought to an end by reason of the very small proportion of water present.

It is easy enough to talk about water—we have a very fair knowledge of what we mean by the term, and although we suspect now that water is not a mere agglomeration of molecules of H_2O, but consists of a number of reversibly convertible species, at least it is one substance and under any given set of external conditions it will always behave in the same way.

When we come to protein, it is to be hoped that some surprise will have been occasioned that anyone should have had the temerity to lump together a large number of highly specialised single individuals under the designation of a single substance. But although it has been urged on other occasions (Jordan Lloyd, 1929) that the individuality of the different proteins is the chemical basis of the different plant and animal species, yet on this occasion it will be convenient to regard all proteins together and deal only with their common characteristics as organic colloids. This proceeding is not so paradoxical as it would appear at first sight, because all proteins may be regarded as bodies made up of a backbone and limbs, and their colloidal properties, which are common to the group, lie in the backbone, while their chemical properties, as well as those colloidal properties which distinguish them as individuals, are found in the limbs. Therefore, just as it might be possible to discuss the anatomy of the backbones of the vertebrate animals and ignore for the time being the differences in the limbs in fish, amphibians, reptiles, birds and mammals, so it is possible here to discuss the backbone of proteins and its fundamental influence on the properties and behaviour of the different biological tissues.

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[1] This paper was read before the Biochemical Society of the University of Birmingham on November 5th, 1931. My thanks are due to my colleague, Dr H. Phillips, for considerable assistance and valuable criticism in preparing the paper for the press.

A few words now on the chemical structure of the protein backbone. This may be represented diagrammatically in the conventions of classical chemistry as shown in Fig. 1. This backbone can be regarded as a string of vertebrae or segments, each consisting of a short 3-atom chain formed by 1 nitrogen and 2 carbon atoms and joined to its neighbour by a peptide link. Since octa-peptides are the simplest peptides giving typical protein precipitation tests, we can consider that the backbone must have at least eight segments. In the diagram we have just been considering, the string of carbon and nitrogen atoms has been drawn in a straight line, but this is not a likely arrangement in space. Modern views on the orientation of valency bonds suggest that the atoms are probably orientated round a spiral, and in that case their projection on to the plane of the paper will be more accurately represented by a zig-zag line, the angles of which are at approximately 120° (Fig. 2).

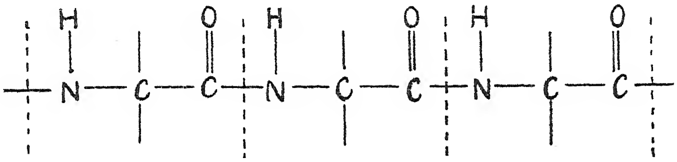


Fig. 1.

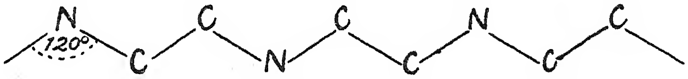


Fig. 2.

There are two points to notice about this protein backbone, which for convenience may be designated by the letter P. The first is that the terminal segments are differentiated from the others, since obviously they can only be connected with a peptide link on the one side. This leaves at the two ends of the chain respectively, a carbon and a nitrogen atom, each with a co-valency bond to be satisfied in some other way. In the case of the carbon atom, the co-valency bond is satisfied with a hydroxyl group which, with the ketonic oxygen already present, builds up a carboxyl group as the terminal group of the molecule. In the presence of water this group ionises as $(P.COO)^- + H^+$, the separation of the positively charged hydrogen ion leaving a negative charge on the end of the long protein molecule (P). In the case of the nitrogen atom, the co-valency bond is satisfied for trivalent nitrogen by linking up with a hydrogen atom, which with the hydrogen atom already present leads to the formation of an amino group ($-NH_2$). In aqueous solution, however, the trivalent nitrogen of an amino group is not stable and the nitrogen becomes pentavalent and linked up with the positive and negative elements of water, so that the terminal amino group of a protein molecule has, in solution, the form $P.NH_3OH$, or, since ionisation occurs, $(P.NH_3)^+ + OH^-$. This ionisation leaves a positive charge on the end of the long protein molecule. The protein molecule, therefore, is polar and has the character of a Zwitterion. It can be written in the form $OH^- + {}^+(NH_3.P.COO)^- + H^+$. In solution, because of their polar character, the head and tail of the protein molecule will be hydrated.

The second point to notice about the protein backbone is that every segment carries the possibilities of attachment of four limbs by means of co-valencies. We know a little about the nature of the limbs of the protein molecule and we have a certain amount of evidence about their orientation round the backbone. The two limbs which are carried by the nitrogen and terminal carbon atoms of each segment are fixed in character and position by the nature of the peptide linkage. They are a hydrogen atom and a double bonded or ketonic oxygen respectively (see Figs. 1, 2). Analogies from organic chemistry suggest that it is highly likely that the ketonic oxygen, which is on the terminal carbon atom, shares an electron with the hydrogen atom of the nitrogen and thus forms a 5-atom ring on each segment, one of the most stable forms known in organic chemistry (Fig. 3). The third and fourth limbs are both attached to the centrally placed carbon atom. The third limb is always a hydrogen atom. The fourth limb may be one of a large number of chemical groups—it may be a hydrogen atom, a methyl or a hydroxymethyl or, in fact, any of the normal or iso-forms of the lower hydrocarbon groupings containing up to four carbon atoms. It may, however, be more complicated and contain ring structures—benzene, indole or imidazole rings. It may also be basic in character from the presence of an amino or a guanidine grouping, or acid from the presence of a carboxyl grouping. It may even be a grouping containing sulphur. The variable fourth limb is always on the middle carbon atom of the 3-atom chain that forms the unit. Except when the fourth limb is a hydrogen atom, this middle carbon atom is asymmetric and gives rise to optical activity whether the series of segments is joined up into the intact backbone of the protein molecule or whether the protein has been hydrolysed and each segment released in the form of its corresponding free amino acid. The optical activity of the amino acids has received considerable study (see Clough, 1918; Fischer and Raske, 1907, 1908; Karrer and Kaase, 1919, 1920; Waser and Brauchli, 1924), and the conclusion has been reached that all the naturally occurring amino acids (*i.e.* those derived from proteins) have the same spacial configuration, or, in other words, that the four groups, R, H, COOH and NH_2, always lie in the same relation to each other and to the asymmetric carbon atom to which they are attached by co-valencies. Of course, this does not mean that all naturally occurring amino acids cause optical rotation in the same direction. Whether an amino acid is laevo- or dextro-rotatory depends on the constitution of the R

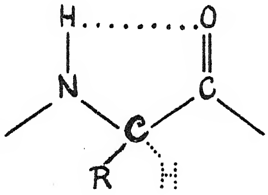
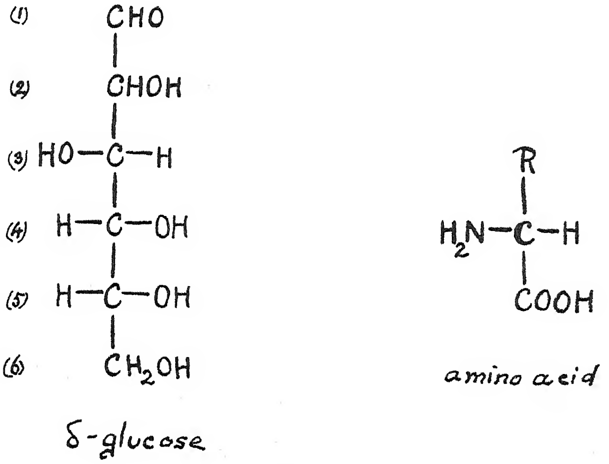


Fig. 3.

group. The actual placing of the four groups in 3-dimensional space round the asymmetric carbon atom is not known for the amino acids any more than it is known for any other optically active carbon compound. However, the actual placing in any one optically active substance does not seriously matter, provided one can always be sure of the relative placing in comparing one substance with another. Convention has agreed that the fourth carbon atom of *d*-glucose shall be taken as the arbitrary standard to which the spacial configuration of all optically active carbon compounds is to be referred and it has been found that the naturally occurring amino acids must be considered to possess the opposite configuration to that assigned to this arbitrary standard. The placing of the groups in *d*-glucose and in the amino acids on the conventional plane diagram is shown in Fig. 4.



δ-glucose

amino acid

Fig. 4.

Neither the molecule of glucose nor the molecule of an amino acid is, however, regarded to-day as lying in a plane; the glucose molecule is known to consist of a 6-atom ring with the hydrogen and hydroxyl groups lying above or below the plane of the ring (see p. 271), and 3-dimensional models of amino acids show that here also there is a group of five atoms lying in one plane with the "R" group and the hydrogen atom attached to the asymmetric carbon atom lying above and below this plane respectively. Comparing the structure with that of glucose (see Haworth, 1929), the deduction can be made that if the 5-atom ring be drawn with the atoms N, H, O, C, C in clockwise order, then the "R" group must lie above, and the H group below, the plane of the ring (see p. 265). In the diagrams shown in this article, atoms drawn in with a dotted line are to be taken as lying below the plane of the paper.

This is an important point in protein structure. If one builds up a protein molecule from models, two possibilities appear: in the first one, the ketonic oxygens are all on the same side of the backbone. This is not a simple zig-zag; it shows a definite tendency to come round in a circle so that the two polar groups lie close together, giving the possibility of internal salt formation. In this model, all the "R" groups lie in a plane either above or below that of the main axis. A projection of this model on to a plane and pulled out to lie along a straight axis is shown below (Fig. 5). On account of its circular form, this model does not suggest a structure which would lead to close packing, but one which might be assumed by cell proteins because it is limited in size and because the large, highly polar groups, which such proteins contain, could easily be built into it. The other possibility is shown below (Fig. 6).

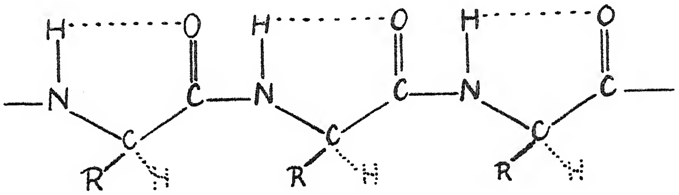


Fig. 5.

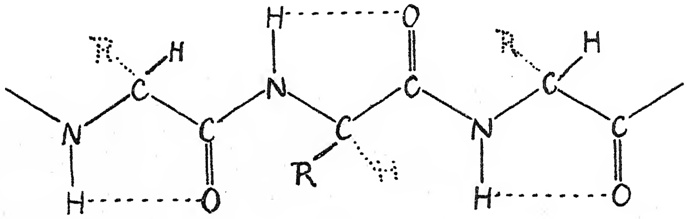


Fig. 6.

Here, by the simple device of rotating alternate segments, we get our zig-zag backbone, which can be extended indefinitely. In this model, the "R" groups lie alternately above and below the plane of the main axis, an arrangement which, as we shall see below, opens up possibilities of very close packing with elimination of water. This second possibility of structure, each molecule having its side groups alternately right and left, lends itself readily to making models of fibres. It is very interesting in this connection that Murat and Edsall (1930) consider that even a protein connected with such a typical example of biochemical activity as muscular contraction, namely myosin, exists in the muscle cell in the form of rods.

We may, therefore, regard a protein as a substance which has a backbone which is chemically stable and limbs which have possibilities of chemical activity. These limbs may also occupy a good deal of space round the backbone, a matter of considerable biological importance.

There is also another point about such protein molecules which seems to be the very essence of the structure of living colloids. Put very simply, it is this—that the backbones of the protein molecules in solution are not highly solvated but the limbs and the head and tail may be, in fact generally are, in the circumstances we are considering here. This ingenious arrangement gives physical stability with chemical activity, which is what is wanted in protoplasm.

We might perhaps with advantage spend a few moments considering what is meant to-day by the term "solution." The subject has been the centre of an enormous amount of research since the days of Faraday, but modern views come to this—that wherever solution occurs there is localisation and orientation of the molecules of the solvent round those of the solute, and that, as a result, the total activity of the solvent becomes reduced in amount. The solute with its adhering layer of solvent molecules is described as solvated.

In our protein molecule, the backbone is largely protected from solvation by the non-polar properties of its own atoms and also by the positions of the limbs, and, therefore, even in so-called protein solutions, the backbones of the molecules may be regarded as a non-aqueous phase of atoms occupying fixed positions. The two polar groups at the head and tail are, of course, solvated and so are the limbs in varying degree, according to their chemical constitution. The solvation of the limbs will increase the amount of space they occupy.

So much for the chemistry of the proteins as a single class of substances. Their chemistry as individuals and the biological significance of their individual chemistry is of immense importance, but we are leaving it aside on this occasion. For our present purpose we are only considering the biological significance of the colloidal structure which is common to them all.

This brings us really back to the main subject of this discussion, namely, the relation between the protein of protoplasm and the water in cellular structures and of the biological significance of this relation. We may consider the cells and tissues of organisms under two heads, namely, (1) active metabolising cells and (2) non-cellular structures not concerned with metabolism, namely fibres.

Cells. It has been shown on a number of occasions that young organisms contain more water than old ones. This is partly due to the separation and development of structural tissues, such as the skeletal tissues of plants and animals, and partly to the fact that the cells concerned in the actual metabolic cycle contain less and less water as the organism passes from childhood to maturity and then on to old age. The subject has recently been dealt with very thoroughly in Needham's *Chemical Embryology*, from which Figs. 7 and 8, shown below, have been taken.

Fig. 7 shows the percentage of water in the developing chick embryo. It shows that the water content of the embryo first rises and then slowly falls away. In a personal communication, Dr Needham has stated that he has other evidence which shows that the period of greatest activity in the embryonic development is about the fourth and fifth days, which is also the wettest stage of the embryo's development. The embryo, of course, consists not only of water and protein but also of carbohydrate, fat, inorganic salts, etc. If we consider the relation of the water in the embryo to the protein present, the dehydration that occurs during development is even greater than is shown in the figure, since at the fourth day 73 per cent. of the dry weight is protein, whilst at hatching only about 55 per cent. is protein.

Fig. 8 shows the percentage of water in mammalian embryos. The curves shown are for man and a number of the domestic animals, and it can be seen that all the embryos lose water as they develop. The details of the figure are not of any great significance to us, but it is interesting to note that what is true of the embryo as a whole is equally true of each organ, say, for instance, the developing lung, which has been studied in great detail in the sheep.

If the biological activity of cells can be associated with their content of water, it is important not only to survey the embryo or the adult as a whole, but to consider the different organs individually. Cramer (1916) has made an estimate of the water content of the various organs of 8 weeks old male mice. His analytical data are summarised in Fig. 9, which shows the water content of muscle, heart, liver, kidney, spleen, testis. It is very striking how much more water content there is in the testis than in any other organ and therefore here again we find active cell division and growth associated with high water content. It has been suggested that the greater amount of water found in young tissues is due not so much to the fact that the individual cells contain a greater proportion of water to protein, as to the fact that the tissue as a whole contains a greater proportion of the primary connective tissue, which, it is agreed by everyone, is the wettest tissue in the body. A convenient supply of primary connective tissue is to be found in tumours, and Fig. 9 also gives figures for the water content of a number of tumours in 8 weeks old mice. The water content is considerably higher than in any of the tissues of the body of the mouse and,

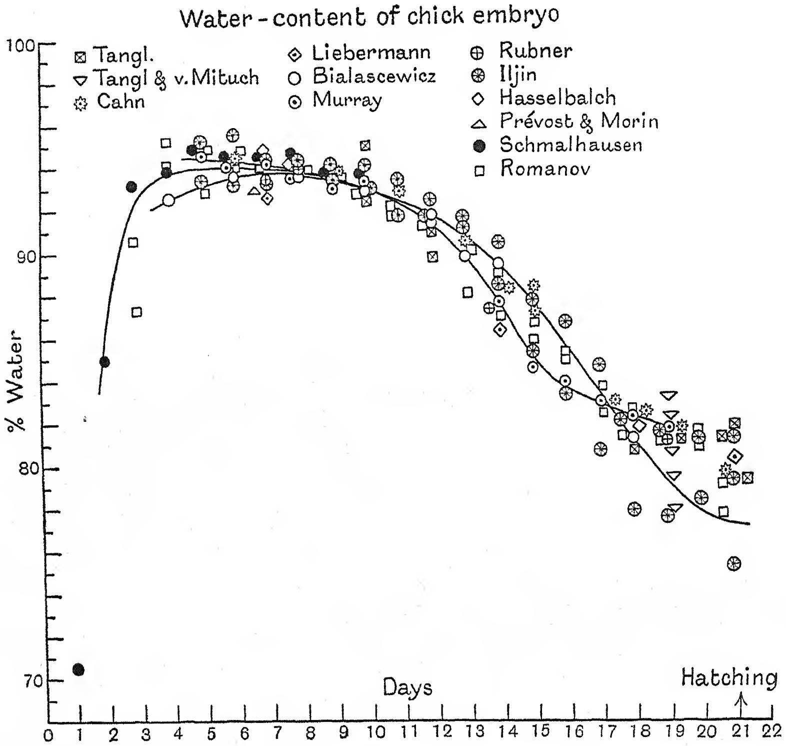


Fig. 7.

moreover, the more actively growing tumours contain more water than the less actively growing ones. The growth rate of the tumours is indicated by the blackened portion of the diagram. In addition, the same relation as regards protein holds as with embryonic tissues—the more actively growing tissues contain rather more protein in their dry weight than the others.

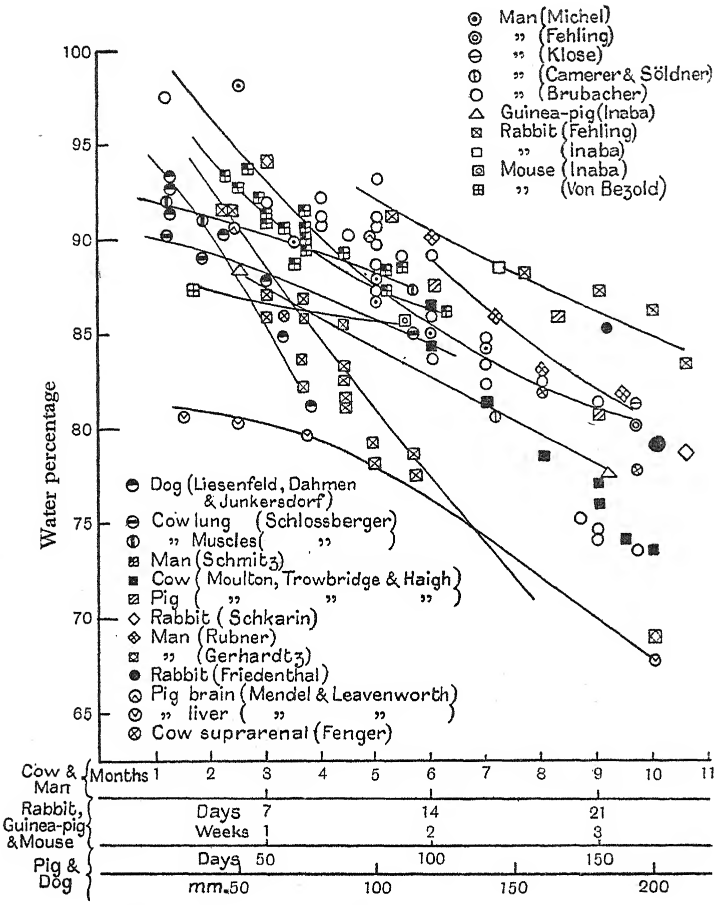


Fig. 8.

It is interesting that this relation between cell activity and the state of the proteins also occurs in those animals in which a physiological gradient can be demonstrated. The earthworm can be taken as an example—the highest potential of physiological activity is at the head end, the next highest at the tail end and the lowest in the middle section. Ruzicka (1927) has shown that this physiological gradient has a parallel in the state of the cell proteins—these are most highly dispersed and hydrated at the head end, and least highly hydrated and dispersed in the middle section, the state of the proteins from the tail end being intermediate. Ruzicka uses the precipitability by alcohol as a measure of dispersion and hydration. He does not give the percentage water content or the percentage protein content of his different regions, but there seems little doubt that they would fall in the same order as the physiological activity, *i.e.* the head end would have the highest percentage of water and the middle section the least.

Consider now the chemical conditions in the metabolising cell. An important point to emphasise here is that the molecules involved in any chemical reaction must occupy space and that very few of the chemical reactions which occur in cells and give a supply of energy for the work of the cells involve the protein molecule in any way. Therefore, within the cells there must be available enough free water for these chemical reactions (which, it may be assumed, only take place if the interacting substances are in solution) to occur. That living protoplasm has free water available is easy enough to demonstrate. A *Paramecium*, for instance, may be seen to secrete vacuoles round the particles of food which it has swallowed and to reabsorb these vacuoles when digestion is complete. Gland cells in a state of activity become swollen and vacuolated. That the water available for chemical activity is, however, limited in amount is probable from the high viscosity of the protoplasm (see, however, Moran and Smith, 1930). The availability of the water can be measured by the ease with which it can pass into or out from the cytoplasm which can be regarded for our purpose as a hydrated or emulsoid colloid in solution in water.

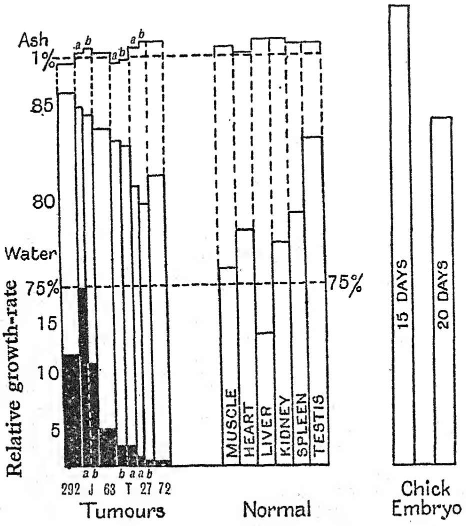


Fig. 9. 292, J, 63, T, 27 & 72 are strains and *a* and *b* generations of strains.

The problem is easier to study indirectly than directly, and many non-living models for the experimental investigation of the passage of water in and out of protein systems by observations on osmosis, swelling and so on, are recorded in the literature. The only study of this sort which need be considered with any detail here is some recent work on the influence of gelatin concentration on the behaviour of gelatin jellies (Northrop, 1927; Jordan Lloyd, 1931). It is familiar to everybody that for a gelatin jelly in contact with an aqueous solution, the amount of water in the jelly depends on the *p*H value of the system and the concentration and nature of all the electrolytes present. It is also influenced to a remarkably high degree by the concentration of the gelatin at which the jelly was set in the first place. Fig. 10, taken from the paper by Jordan Lloyd, illustrates how this influence of the initial concentration shows itself whether the jelly is in equilibrium with water or solutions of acids, alkalies or electrolytes and over the whole range of temperatures at which the jelly can maintain its physical integrity. Under all conditions, swelling is a function of 1/log *G*, where *G* is the percentage concentration of the jelly at the time of swelling.

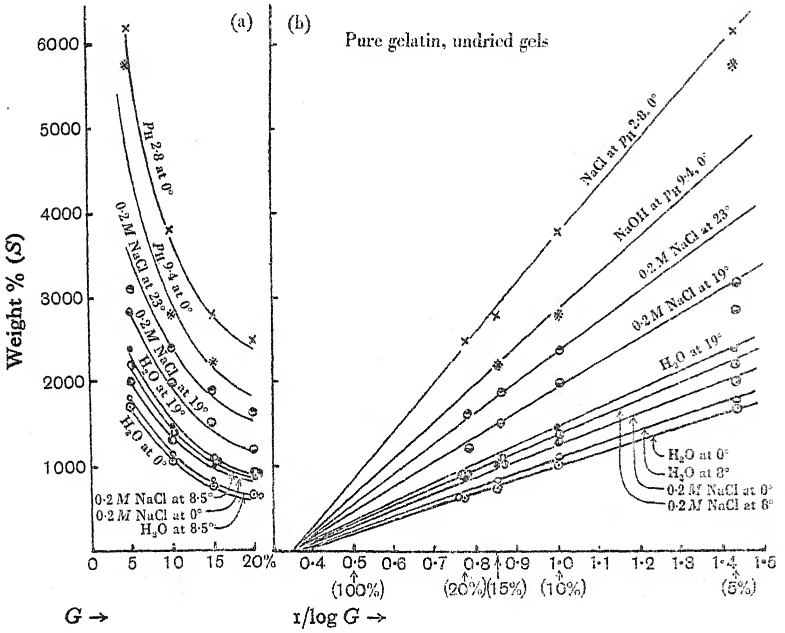


Fig. 10.

The important feature of these curves from the point of view of the relation of biological activity to water content is that the jellies containing the lowest percentages of gelatin show the biggest changes in response to changed external conditions, *i.e.* the ease with which the water passes into and out of the jelly, or the availability of the water, is an inverse function of the concentration of the gelatin at setting.

Many analogies have been made from time to time between the behaviour of simple models, such as gelatin jellies, and that of tissues. Many of these have thrown much illumination on the physical chemistry of the living cell, but it is always a little difficult to know how far an analogy may be pushed with safety. However, it has been postulated in biological literature that protoplasm can exist either as a sol or as a gel and that at certain stages in the life history of a cell there may be transformation from the sol to the gel state or *vice versa*. Wintrebert (1931), for instance, considers that the unfertilised egg of an amphibian *Discoglossus* possesses rigid properties which he ascribes to the presence of a cytoplasmic skeleton which he appears to suggest is of protoplasmic origin, though without discussing whether it is protein in nature. This cytoplasmic skeleton apparently disappears after the fertilisation of the egg and before cell cleavage begins. A review of further literature and a discussion of the problem will be found in Gray's *Cytology*. However, granting this assumption for the moment, we do get from it an idea of the physical basis of senescence in cells, for there is undoubtedly a general tendency for water to drift out of the cells and tissues as the organism grows older, while the content of protein or other colloids remains the same. A sol-gel transformation occurring periodically in the course of this continuous drying up process would lead, with every reformation of the gel stage, to a change in gel structure corresponding to the gradual decrease in the water content of the cell. This colloidal model of the process of "ageing," though admittedly crude, does give an explanation as to why the cell protoplasm becomes less and less responsive to external conditions and why the availability of the water for chemical reactions also becomes less and less.

There is even a colloidal model to account for the automatic loss of water with time, for Kunitz (1928) has shown that if gelatin jellies are made by dissolving different amounts of gelatin in distilled water, the jellies may be made of any concentration from 1 to 20 per cent., but although they are all, so to speak, perfectly good jellies, the only one that is stable is the 10 per cent. jelly. The weaker jellies are automatically self-concentrating; they give up water either in air, a process long known as "synaeresis," or when immersed in water. The stronger jellies placed in water are self-diluting, *i.e.* they take in water. The weak jellies, therefore, exhibit the phenomenon of senescence. If they are made and kept under water they will become less and less responsive to changed external conditions with the passage of time.

This loss of sensitiveness may reasonably be regarded as due to nothing more or less than a drawing together and closer packing of the gelatin molecules. The mere condensation of these leads to a diminution of the space available for molecules of water and other substances.

The colloidal structure of biologically active protoplasm may, therefore, be visualised as follows: the protein molecules which are the basis of the protoplasm, are elongated particles possessing a certain degree of rigidity conferred upon them by the carbon-nitrogen chains which forms the backbones of the molecules. These molecules are probably orientated at any interface, such as a cell surface or the wall of a cell vacuole, while they lie criss-cross in the body of the cell. Such an arrangement would lead to a high degree of stability of structure. The protein molecules also possess side chains or limbs which may be short or long and which may be hydrated and therefore regarded as in solution in the water present. Moreover, there is some free water capable of entering into solution with other substances and thus available for the chemical activities of the cells. In youth, the protein particles are separated by a large amount of water, but as time passes by the protein particles draw closer together under the action of their own forces and water is lost from the cell. With this loss of water there is a loss of physical response and chemical activity.

Ruzicka and his pupils have carried out some very interesting work which brings definite evidence to bear on this point. Berganer (1927), for instance, has shown that the percentage of protein in ox serum rises from about 8 per cent. at birth to 10 per cent. at 15 years of age, and that this increase in the protein content of the blood is accompanied by a fall in the albumin percentage with a rise in the globulin percentage—in other words, not only is the blood as a whole drying up, but the more highly hydrated albumin is being replaced by the less highly hydrated globulin. There has been much discussion from time to time as to the relation of the albumin to the globulin of the serum, some workers holding that globulin is derived from albumin by a kind of mild degree of denaturation and others pointing out that the two proteins have a different constitution, and arguing, therefore, that one cannot be derived from the other by any change in their physical state. That is, of course, quite true, but when we look at the chemical constitution of the two proteins, we find that they only differ in two points in their amino acid constitution: serum albumin yields 13 per cent. of lysine on hydrolysis while globulin only yields 9 per cent., and albumin yields no glycine while globulin yields 3 per cent. of glycine. In all other respects, the amino acid constitution is the same (see Jordan Lloyd, *Chemistry of the Proteins*, for reference to original data). It is, therefore, only necessary to remove about a quarter of the lysine limbs of albumin, which we know from other evidence are always terminal, leaving a hydrogen atom to fill up the vacant co-valency, and we pass directly from albumin to globulin. The terminal amino group of lysine is a negative group, *i.e.* it is polar and hydrated. The removal of the lysine side chain, therefore, would mean loss of polar groups and reduced hydration, and would be enough in itself to account for the lower solubility and greater tendency to coagulation of the globulin (Fig. 11). This scheme of colloidal behaviour, *i.e.* a loss of polar groups and consequent dehydration leading to an increasing aggregation of the protein particles, leads us on not only to the consideration of senescence in cells

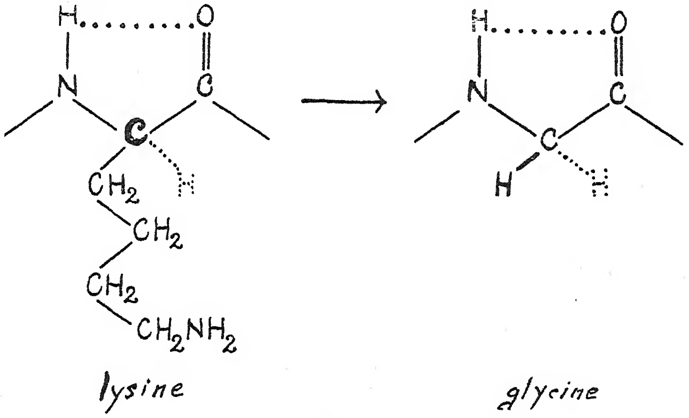


Fig. 11.

but also to a consideration of another class of structures found even in young organisms, namely fibres.

Fibres. The densest state of packing of colloidal molecules is found in fibres, and a striking instance of the close correlation between colloidal structure and biological function is revealed by an examination of the distribution and function of fibres in animals and plants. In making this survey, however, it is important to notice that those very important tissues, muscles and nerves, are excluded; muscle fibres are not, in the general sense of the term, fibres at all—they are active elongated nucleated cells and the same is true of nerve fibres. True fibres are elongated products of cell activity; they are not nucleated nor connected directly with any vital activity. They are mostly remarkably resistant to bacterial putrefaction, a matter of considerable importance when considering both their structure and their function. Speaking generally, animal fibres are made of protein and plant fibres of carbohydrate, a contrast in building material undoubtedly due to the fundamentally different types of metabolism in the two groups of living organisms.

In particular we can consider animal hairs and connective tissue fibres and the cellulose fibres of plants, such as bast fibres and cotton hairs. Although connective tissue and hair are of protein nature and of animal origin, while bast fibres and cotton are plant structures formed from carbohydrate material, when the condensation and packing of the colloidal particles is sufficiently dense their chemical nature has little influence on their properties. This apparent paradox presents no difficulty, for, since biochemical reactions can only occur in aqueous solution, it is only necessary to squeeze out the water in order to bring them to an end. The importance of fibres in both plants and animals is their chemical and physical stability.

Connective tissue in vertebrates consists of three kinds of fibres, all of protein nature. They are the reticular fibres formed of reticulin, of which we know nothing except that it is very resistant to chemical reagents; yellow elastic fibres formed of elastin, of which we know rather more; and white elastic fibres formed of collagen, about which we know a considerable amount.

The first and most interesting thing to note about collagen is that if hydrolysed into its separate amino acid units, about a quarter of the molecule is found to be made up of glycine. This is the simplest of all the amino acids, being the one in which the "R" group is a hydrogen atom (see p. 265), *i.e.* the smallest of all the groups ever found occupying this position. If, therefore, we have a long peptide chain made by condensing glycine molecules, there will be no difficulty in bringing it close up against a similar chain.

Fig. 12 shows three long polypeptide molecules laid close together. The middle one has been placed running in the opposite direction to the upper and lower, thus bringing the imino-nitrogen from the one molecule opposite the ketonic oxygen of the adjacent one. With such an orientation there would be an attraction between adjacent chains (see also Astbury and Woods, 1931). If a model such as that shown in Fig. 12 is constructed in three dimensions, it will be noticed that the "R" groups of adjacent molecules either both lie above or both lie below the plane of the paper.

Possibilities of cross-attractions, therefore, also occur at the "R" groups if these are polar (see also Speakman and Hirst, 1931).

It is important to notice that this apposition of the chains is pure guesswork and that it would be unsafe to labour the point, since it must be borne in mind that about one-fifth of the molecule appears in the products of acid hydrolysis as proline and hydroxyproline and that both these molecules are much larger than the glycine molecule. Proline is a ring compound, but its precursor in the protein molecule is probably the amino acid δ-hydroxy-α-amino-valeric acid, which has a 3-carbon side chain (Fig. 13) (see Sørensen and Anderson, 1906; Knaggs and Schryver, 1924), while that of hydroxyproline may be δ-γ-hydroxy-α-amino-valeric acid or possibly the ketonic form.

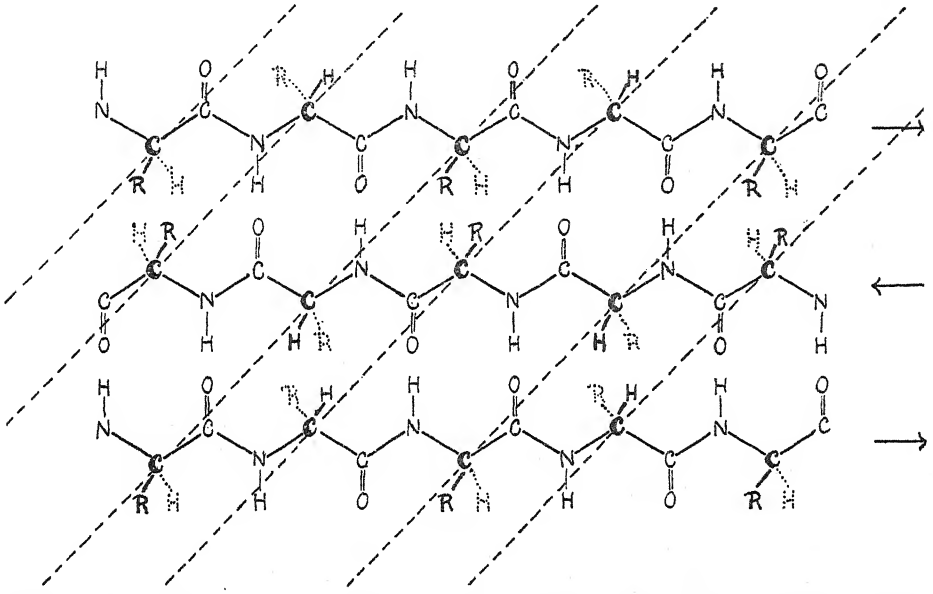
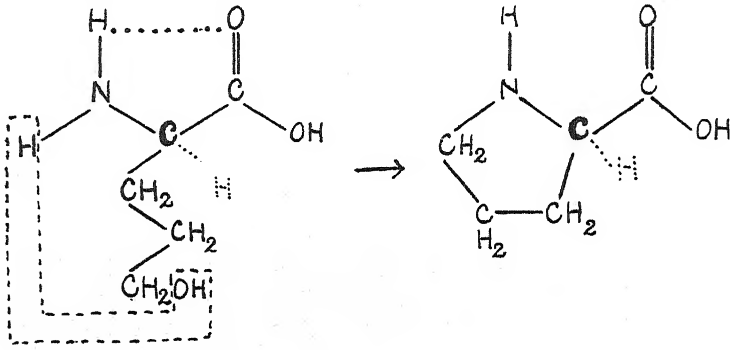


Fig. 12. The solid "H" and "R" groups on the asymmetric carbon atoms lie above the plane of the paper. The dotted "H" and "R" groups lie below the plane of the paper.

There is, however, evidence of quite a different type suggesting that collagen fibres are composed of elongated molecules closely packed and running parallel to the axis of the fibre, namely evidence from X-ray analysis.

Perhaps, at the risk of going over familiar ground, it might be worth while to pause for a moment to consider the physical basis of X-ray photographs. X-rays are to be regarded as electro-magnetic waves of exceedingly short wave-length, so short that they can penetrate through the ordinary coarse-grained structure of a solid object but can be deflected from their course by impact with an atom. The use of X-rays in the analysis of crystals is founded on the fact that in a crystal the position of every atom is fixed, and every crystal is made up of the orderly repetition of crystal "units." This unit may involve one or several molecules. All units are alike and similarly oriented. The crystal, therefore, has a regularly repeated pattern and just as a wall-paper or any other regularly patterned plane surface has the individual points of its unit pattern lying in regular lines, so a regularly patterned 3-dimensional solid body has the individual points of its unit patterns (namely the atoms) lying in regular planes (see Fig. 12). These planes form as it were internal reflecting mirrors for the X-rays and from the pattern reflected from these planes and recorded on a photographic plate it is possible not only to obtain information about the crystal units but even also about the atomic pattern of the unit.

But to go back to collagen—collagen has an X-ray pattern, but gelatin, which is derived from collagen (either by heating with water under pressure, or by treating the collagen with an alkali and then neutralising and extracting with warm water), has no X-ray pattern. On the other hand, if gelatin is cast into a jelly, the sides or ends of which are fixed, and then this jelly is dried so that mechanical strain is set up, then this strained gelatin is now found to show an X-ray pattern which is very closely similar to that of collagen (Gerngross and Katz, 1926; see also Abitz, Gerngross and Herrmann, 1930).



δ-hydroxy-α-amino valeric acid proline

Fig. 13.

What does this mean from the chemical standpoint? It means that collagen and gelatin both consist of the same protein and that this protein is built up with a regular, repeated atomic pattern. We know, from other evidence, that the gelatin molecule can be regarded as an elongated particle. In gelatin, either as a sol, a gel or a dried horny mass, these elongated molecules can be regarded as lying without orientation, like a pile of spillikins, but in gelatin dried under strain, a parallel orientation of the molecules will have been brought about by mechanical forces. In collagen, orientation will have been brought about by the delicately controlled activities of the living fibroblasts which laid down the collagen fibre.

We can now see that gelatin can provide no internal mirrors to reflect X-rays but that strained gelatin and collagen can do so. The arrangement of the long polypeptide chains shown in Fig. 12 leads, for instance, to all the asymmetric carbon atoms lying in planes in the fibre. The circumstantial evidence, therefore, for the parallel orientation of the elongated molecules is strong.

The microscopical evidence on the structure of collagen fibres is very interesting. Those from the skin, for example, run in fibre bundles; each bundle consists of fibres, and by suitable treatment each fibre can be seen to consist of fine fibrillae all running with their axes parallel. In considering the fine structure of collagen fibres, it is not a little entertaining to discover that if gelatin is dried in the strained condition and then struck a sharp blow with a hammer, it also breaks up into a large number of fine fibrillae, all arranged with their axes parallel to the direction of strain (Katz and Gerngross, 1926).

Let us now consider collagen fibres from the biological standpoint. We find that their biological properties are exactly what we should anticipate from their chemical structure. They occur in the body, in the skin, in the mesenteries and other connective tissue sheets and in tendons. There is no evidence from the biological side that the white fibres of connective tissue, the collagen fibres, have any biological activity whatever. Their biological functions are purely mechanical and the chief thing required of them by the living body is that they should not indulge in biochemical activities but that they should just "stay put." But how is the body going to ensure unchanging behaviour on the part of a tissue which is continually exposed to contact with that biologically complex fluid, the lymph? What better way could be devised than by taking a protein molecule, knocking off its soluble polar side chains or limbs and bringing its backbone sufficiently close to others of the same kind to squeeze out water and all the chemically active substances which it contains in solution. How successful the body has been in constructing a substance with valuable mechanical properties and no biochemical activity anyone who has ever torn a tendon in their ankle joint will know only too well.

Collagen fibres are not, however, carried to the extreme of spacial condensation of which the body is capable. They are still capable of reacting with acids and alkalies and showing the phenomenon of colloidal swelling, *i.e.* it is possible to drive water in between the backbones of the individual molecules without disrupting the whole fibre. Although collagen is not attacked by the proteolytic enzyme trypsin, it is capable of hydrolysis by pepsin. The fibre, therefore, is sufficiently condensed to exclude many biochemical reactions but not sufficiently to exclude all possibilities. Collagen fibres are, after all, internal structures, and too great a degree of stability might have its disadvantages.

The extreme of colloidal condensation is not found in this internal protein but in one which only occurs in structures which are external to the body, namely the keratins, products always of epidermal activity.

The structure of the keratin molecule has been investigated by the method of X-ray analysis by Ewles and Speakman (1930) and by Astbury and Street (1931), who have worked largely on wool. Astbury (1931) finds, however, that many keratinous structures, *i.e.* all animal hairs and even horn and finger nails, give the same X-ray photograph. In the molecular and crystallographic sense, therefore, these keratins have the same fundamental atomic pattern. They have not all the same chemical composition and the analyses available are not sufficiently good to allow of very sweeping generalisations as to the relative volumes and polarities of the side chains. In wool fibres, the di-basic acids lysine and arginine, together with the di-carboxylic acids, make up roughly a quarter of the molecule; in hair and horn, the proportion of these highly polar side chains appears to be less and in all the keratins complicated rings are scarce. On the whole, therefore, there would appear to be no difficulty in making a compact fibre out of the keratin molecule. Astbury's work on the X-ray diagram of keratins suggests that these may be the most compact of all protein molecules. This compactness, according to Astbury, has been achieved in a remarkable way, namely, by folding over the long polypeptide chain into a series of open rings (Fig. 14).

It is well known that mere mechanical tension can pull out wool and hairs to nearly double their original length (see Speakman, 1929). With the stretching there is a change in X-ray diagram that Astbury explains by assuming that the molecules have been pulled back into the zig-zag line of the typical polypeptide chain. Astbury calls these stretched keratins "β-keratins," and he has shown that the molecules can be fixed in the β-position by steam or weak solutions of sodium sulphide. Speakman, however, who has also been working on the structure of the wool fibre, does not appear to consider it necessary to invoke the folding of the polypeptide chain though he does consider that the wool fibre is built up of unit plates impervious to water (1930).

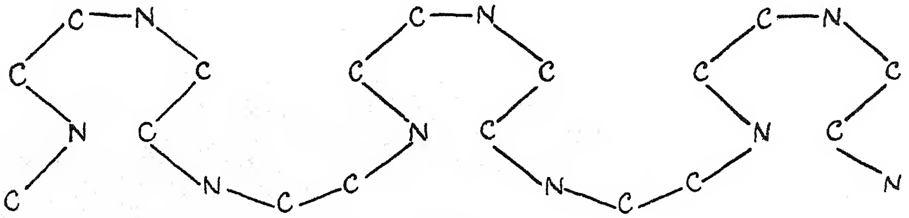
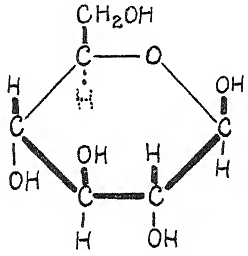


Fig. 14.

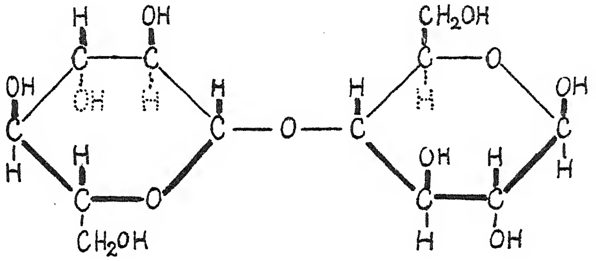
It is easy to see how the compact structure suggested by Astbury could form the basis of the water-free and, therefore, chemically stable units. The biological properties of the keratins are exactly what one would expect from such a structure. To begin with, they are resistant to all the proteolytic enzymes. The peptide links in the compact molecules are less accessible to the enzymes. The whole of the outer layer of all animal bodies is covered with a keratinous layer which, therefore, shields the body from bacterial attack from without. Keratinous structures such as wool and hairs are hygroscopic and they swell or shrink to a certain extent by the passage of water in and out between the impervious unit plates. Their capacity for water absorption is, however, very small compared, say, to gelatin and they undoubtedly protect, for instance, a swimmer from absorbing water while swimming in a freshwater stream or losing it while swimming in a brine bath. The keratins also are resistant to the hydrolytic action of dilute acids and even to a considerable degree to that of dilute alkalies. In short, in both physical and chemical properties and biological functions, they stand apart from all the other proteins. They are, however, true proteins and the recent work with X-rays has shown that their special characteristics are due to a variation on a pattern which is fundamentally unaltered, *i.e.* keratins, like other proteins, are built up of a unit tri-atomic segment indefinitely repeated.

The keratins are a class of substances peculiar to the animal kingdom. Plants make themselves a protective cover for the protoplasm of their cells out of an entirely different chemical substance. The green plant has the power of synthesising glucose from water and carbon dioxide under the action of sunlight. Since water, carbon dioxide and sunlight are generally available all together during the spring and summer months, the continual synthesis of glucose presents no difficulty to the plant and this carbohydrate, therefore, certainly forms a convenient raw material not only for cycles of biochemical activity but also for structures which are end products of a non-reversible series of chemical changes such as are involved in building up the permanent stable structures in plants, cellulose cell walls, fibres of the wood or bast, cotton hairs and so on. Haworth (1929) has shown very convincingly that glucose exists commonly as a 6-atom ring (the pyran ring) with a side chain (Fig. 15). The glucoses or pyranoses have, therefore, a compact structure but plenty of polar groups available for becoming associated with the water and for taking part in chemical reactions.



glucose

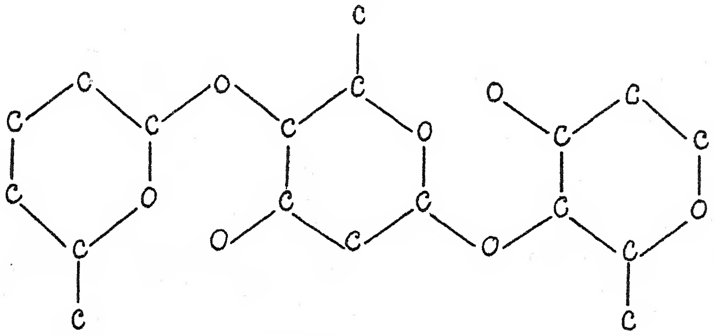
Fig. 15.



cello-biose

Fig. 16.

The glucose molecules have also the capacity for joining up with each other by means of glucosidic linkages and we can get first a disaccharide, cellobiose (Fig. 16). We can, by repeating this, get a long chain of pyran rings joined by glucosidic



cellulose

Fig. 17.

linkages, giving a chain of open structure, and finally by telescoping the chain, *i.e.* by rotating it at the glucosidic oxygens we get a compact chain of this type, which the evidence suggests is probably found in cellulose (Fig. 17).

It is interesting to compare this compact carbohydrate chain of cellulose with the compact protein chain in the keratins (Figs. 14 and 17). Both chains contain a compact backbone formed by regularly repeating units. In both, this backbone consists of 6-atom rings alternating with other 6-atom rings in cellulose and 7-atom rings in keratin. Both keratin and cellulose give a definite X-ray picture showing that they are crystalline in structure. Both contain very low percentages of water. Both are very resistant to chemical reagents and only drastic treatment with boiling strong acid breaks them up into their component units—amino acids in the one case, glucose in the other.

When we consider their biological functions, we again get similarity—the chief function of both keratin and cellulose is protective and therefore it is not surprising to find that both are resistant in a high degree to the attack of the ordinary saprophytic organisms present in air or water.

SUMMARY

To sum up the evidence which we have considered here on the close relation between colloidal structure and biological function, we find that we pass over a series of possibilities. At the one end we get highly active tissues such as primitive connective tissue, growing tissues and generative cells. In these there is a high percentage of water, the percentage decreasing with age; the proteins are present as colloid sols, probably uni-molecular, each molecule consisting of a backbone which is not highly hydrated, carrying at periodic intervals side chains or limbs which are of varied character, often lengthy and of complex chemical structure, highly polar, heavily hydrated and undoubtedly playing an important rôle in the metabolic activities of the cells. Next we get biologically inactive tissues such as mesenteries, tendons and skin, which are built up mainly of connective tissue fibres which have a skeletal but not a metabolic function. Here we find a low percentage of water and the protein molecules are arranged in a compact and orderly manner to form fibres; the backbone of the molecule is still the same, it is very little if at all hydrated and carries at periodic intervals the side chains or limbs, but these are now for the most part shorter and of a much simpler molecular structure, less polar, probably less hydrated and playing little if any part in any metabolic cycle. Finally, we get tissues such as the keratinous layer of the epidermis in animals, cellulose cell walls in plants, external fibres, such as wool and cotton hairs, internal fibres such as bast fibres. The biological function of these tissues is purely mechanical; chemical activity is not desired. Here the importance of the backbone of the colloid is paramount; the chemical potentialities of the limbs are of little significance and we find in these tissues, as might have been anticipated, compact, stable ring structures which *leave very little space for the entry of water* or any other disturbing molecule. In the animal world, these resistant fibres are protein; in the plant world carbohydrate. For biological stability either material is equally effective.

ACKNOWLEDGMENTS

Figs. 7–9 from *Chemical Embryology*, Needham, J., Cambridge University Press, by permission.

Fig. 10 from *Biochemical Journal*, xxv, Cambridge University Press, by permission.

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TERRESTRIAL INSECTS AND THE HUMIDITY OF THE ENVIRONMENT

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(With Four Text-figures.)

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I. INTRODUCTORY.

(1) *Scope.*

IN discussing the relations that exist between insects and atmospheric moisture, it is easy to define the scope of one's enquiry in certain directions. Clearly the "insects" will include all terrestrial Arthropoda; in size and habitat they are not unlike insects, they are covered with chitin, and many of them are tracheate. Clearly also the aquatic insects can be excluded, for their gain or loss of water must be quite unlike that of a terrestrial Arthropod. The eggs of insects are not covered with chitin, nor tracheate; they could therefore be considered as a separate problem, which is dealt with very shortly (p. 306). It is perhaps wise to circumscribe the subject in the following way. Primarily we are studying the effect of water as a factor in the external environment: inasmuch as variations in atmospheric humidity may affect the gain or loss of water by the insect, these subjects are considered. This leads to the subject of water balance in general, and here I have had great difficulty in knowing what should be included. It has seemed best, though it is not very logical, to include studies on the water content of the insect as a whole, and to exclude the rôle of water in excretion and in the activity of particular organs. It is not possible within the limits of this article to introduce the reader to the great mass of valuable information which exists regarding the ecology of insects. I regret this, partly because our knowledge of the relations between insects and water is founded on natural history and ecology; partly also because the insect physiologist is now beginning to interpret the observations made in nature. It appears that we have arrived at the physical law which governs loss of water from an insect (p. 284): it is also shown that certain insects are hygroscopic (p. 280). What discoveries could be more valuable to the field worker who is trying to relate the distribution of insects, in time and in space, to atmospheric moisture? Those who desire a fuller account of field observations on the relations between insects and seasons, droughts, wet and dry places, etc., will find a mass of data in Uvarov's (1931) recent summary. He has made a full collection of existing knowledge on microclimates. There are several other interesting subjects which he has dealt with so thoroughly that I feel justified in passing them over entirely: for instance, the work on the production of "wet" and "dry" forms of butterflies; and the evidence that atmospheric humidity may be a factor producing melanism in Lepidoptera.

Within these limits, the amount of available information is surprising. I have been able to incorporate most of it in the text, but a few papers contain information which cannot at present be related to anything else; these papers will be found in the supplementary list of references. May I take this opportunity of calling the attention of the zoologists to the mass of sound work which is carried out by applied entomologists? Their results are accessible, because summaries are published in the *Review of Applied Entomology*.

(2) *Methods.*

It is clear that atmospheric humidity exercises a great effect on the vital processes of many insects. It is therefore essential to control or at least to measure it, even in work in which the relations of the insect to water are not the main object of study. It is surprising how few workers have yet recognised this fact. There is, for instance, much work on the duration of insect life and the length of developmental stages in relation to temperature. On it is based a mass of talk about van 't Hoff's law, developmental zeros, and other topics which appear to be profoundly important. But the original experiments may have been done under widely different, varying conditions of humidity, and if so, they provide an inaccurate basis for calculation. There are many other types of work in which the effect of humidity has been frequently overlooked, though this is not always the case: Davies (1928) undertook work on the relation of Collembola to moisture, because he thought that this was an essential preliminary to studies on chemotropism; the work of Lehman (1930) on the toxicity of insecticidal vapours was done under controlled conditions of temperature and humidity; the same is true of Brindley's (1930) biometrical work on *Ephestia* (Pyralidae, Lep.) and *Tribolium* (Tenebrionidae, Col.). Lindgren and Shepard (1932) have shown that the concentration of certain fumigants which is toxic to insects is affected by the humidity of the air.

For technical details relating to measurement and control of humidity see Buxton (1931 *c*), and references there quoted: see also Shelford (1929), Smith (1931), Escherich (1930), Tyndall and Chattock (1922), Vernon and Whitby (1931), Bodenheimer and Schmidt (1931), Chattock (1925), Iyengar and Sarathy (1932). Ashbel (1931) points out that certain insect eggs give off ammonia: it follows that if air is passed over them and then through sulphuric acid, the increment in weight is not water (an error which has been made). The problem of hygrometry at the surface of a leaf remains, I believe, unsolved: it might be possible to make some advance with cobalt chloride (see Livingston and Shreve, 1916).

II. GAIN OF WATER.

There are at least four ways in which an animal can gain water. It may do so by actually drinking the liquid: and it must take some water in its food. It is perhaps less obvious that it must derive a considerable quantity of water from the oxidation of its food: finally, there is the possibility that the animal can gain moisture through its surface from a nearly saturated atmosphere.

(1) *The habit of drinking.*

Butterflies. In hot countries, it is a matter of common observation that butterflies visit damp sand or mud and drink from the surface of it, and the same habit has been noticed in Great Britain. References to this subject abound both in papers dealing with the bionomics of butterflies, and also in books of travel. The habit is most conspicuous in Pierine butterflies (Longstaff, 1912; 27 references in index). In many species it appears that the male drinks water and the female does not. Dixey (1907) has published a note on a collection of 153 butterflies which were taken with one sweep of the net at the Ripon Falls, Victoria Nyanza, Uganda, while they were drinking on the surface of wet mud. The specimens were all males, belonging to eight species of Pierinae (*Pinacopteryx* and *Belenois*). It has been noticed that the butterflies are attracted by water which has been fouled by manure (Poulton, 1928); or by damp and sweaty garments (Collenette, 1928). For other facts see Tutt (1898) and Poulton (1916).

Bearing in mind that so many butterflies visit water or else take the nectar of flowers, we may suppose that they are insects which lose water rapidly[1]. Swynnerton (1915), working in Central Africa, found that females of *Papilio dardanus* die quickly in captivity even if they are kept in a large cage with the plant on which they lay eggs in nature. He records that if he held the butterfly in a clip and unrolled the proboscis and put the tip of it in sugar and water, the insect would drink quietly. Females fed in this way survived and laid a considerable number of eggs, and the method was employed on other species belonging to several families. See also Urech (1890), for loss of weight of adult *Pieris*.

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[1] Some Lepidoptera take no food or drink during their adult life. *Philosamia* (Saturniidae) is one of these. Bodenheimer (1931 *b*) gives data about daily loss of weight.

Tabanid flies. It is well known that Tabanidae (Dipt.) drink water. Neave (1911, 1912), working in various parts of East Africa, noticed that these insects drank particularly during the hottest time of the day. Most of them did so by settling on damp sand or mud, but a few of the more slender species appeared to alight on the surface of the water itself. He noted a great preponderance of males among the individuals which drank water. He observed that males of certain species drink the nectar of flowers and the sweet liquid excretions of Aphids. He goes on to suggest that the great preponderance of males at drinking places is due to the fact that they do not drink blood. This explanation may well be correct for Tabanidae, but one must not assume that water can serve as an alternative to blood for other haematophagous flies. Lester and Lloyd (1928) have recorded that if *Glossina* can be induced to drink water it dies immediately. Portchinsky (1915) has published a number of observations made in Russia. He observed that some of the European Tabanidae drink on the wing by skimming the surface of the pool of water, and I have noticed this habit in Palestine. He found that if the surface of the water was covered with kerosene, large numbers of Tabanidae could be trapped. At one pool with a surface of only about 1 sq. metre, he captured 1676 males and 291 females in five days: the specimens captured belonged to several species of *Tabanus* and *Chrysops*.

Bees. The drinking habits of the hive bee (*Apis mellifica*) must be familiar to many people. These insects feed largely on the nectar of flowers, a food which contains a high proportion of water. This they supplement by drinking water, particularly in the spring when there is little nectar, and when they require considerable quantities of water for diluting the stores of concentrated honey. Gendot (1907), who observed bees drinking from the oozings of manure heaps, thought that this liquid was chosen because its temperature was above that of the air. He set out two reservoirs, one at air temperature and the other a little above it, and the bees showed a preference for the second. Many other Hymenoptera have been observed to visit water or wet sand and drink.

Other insects. It is a matter of common observation that certain other insects drink. Muscid flies (*Musca*, *Calliphora*, *Lucilia*, etc.) must be supplied with water if they are to be kept in captivity: cockroaches (Blattidae) also require a supply of water. The beetles *Calandra* (Curculionidae) and *Silvanus* (Cucujidae) breed in dry grain; adults can be trapped in a beaker of water, so presumably they drink (Dendy, 1918); adults of the moth, *Ephestia*, which is also a pest of stored products, can be trapped in the same way. But on the whole, the records of insects drinking appear to be very few, and we are justified in concluding that the great majority of insects obtain their water in other ways. It seems also that many of those which drink use it as a substitute for other liquid foods, particularly for nectar.

(2) *Water taken with the food.*

The substances used as food by insects are extremely varied: they all contain water, but the proportion varies within wide limits. Uvarov (1928, pp. 261–76) has tabulated most of the substances. So far as their water content is concerned, they range from petals and leaves, in which it is over 90 per cent., to apparently dry wax, leather, cigarettes, cereals, etc., in which it is about 5–15 per cent. Many of these "dry" materials are hygroscopic, so that in a moist atmosphere their water content may be considerable. I was able to show (Buxton, 1924*a*) that the dry fragments of vegetation which blow about the surface of a desert contain considerable quantities of water, and that they are hygroscopic if they are put at a relative humidity of 80–90 per cent.: at night, therefore, when the temperature drops many degrees, the fragments of vegetation charge themselves with water. In this way they are a source not only of food but also of water to grasshoppers, harvesting ants, Tenebrionidae, etc. The fact that "dry" organic materials come into equilibrium with the water of the atmosphere in which they are contained opens up a line of work which is hardly yet explored. If one feeds an appropriate insect on bran, feathers, dried blood, etc., one has control of the moisture in its food and also in the surrounding air (see Sikes (1931), whose studies on the larvae of the flea *Ceratophyllus wickhami* suggest, but do not prove, that its successful development depends more on the water content of the food than that of the atmosphere).

Robinson (1928*a*) pointed out that the water content of the food influences the proportion of water in the body of the insect which eats it. For instance, the insects which eat leaves, the water content of which is roughly 90 per cent., themselves contain 82–90 per cent. of water. Those which live on grain, bran, hair, feathers and other materials of which the water content is about 6–15 per cent., generally contain 50–60 per cent. of water themselves. Moreover, these figures hold good for quite unrelated insects. Robinson (1928*c*) has pointed out that the insects living on dry food have a higher percentage of water in them than there is in the food: and he goes on to argue that the increased proportion in the insect must be water of metabolism. It appears to me that any or all of the water may be metabolic, but it may be due to wasteful eating. Indeed, Schulz (1930) has recently discovered that mealworms (larvae of *Tenebrio molitor*, Col.) fed on ordinary bran pass faeces of which 80 per cent. is undigested. This material contains less water than the bran itself, and it appears that they eat wastefully in order to benefit from the small proportion of water rather than from the solids in the bran.

(3) *Water gained from metabolism.*

Several authors have remarked that many species of insects can live on food material containing about 10 per cent. of water, and have suggested that these insects make use of the water of metabolism. Babcock (1912) pointed out that, though oxidation of any stored material must produce a considerable quantity of water, many animals must use it to carry away nitrogenous waste in solution. But those animals which excrete solid uric acid can economise water which would otherwise be used in excretion. He showed that clothes moths, grain weevils and other insects which live on very dry foodstuffs excrete uric acid, and he produced presumptive evidence that they retain and use water of metabolism. But he did not prove that this is actually done. The meal-worm is the only insect of which we can say with confidence that it makes use of the water which results from oxidation of its reserve material (see p. 298).

(4) *Water obtained by absorption from moist air.*

Certain insects can gain water from an atmosphere which is nearly saturated, or from moist soil; the water enters the body through the surface of the insect and not through the mouth. The eggs of some insects possess a similar property (p. 308). Such insects may perhaps be called "hygroscopic" provided that no precise or dogmatic meaning be attached to the term. The best known example is the meal-worm (larva of *Tenebrio molitor*); when this larva has been starved for 2 days, it will lie motionless and inactive even at so high a temperature as 30° C.: it is therefore particularly appropriate for many experiments. If meal-worms are kept at a relative humidity of 80 per cent. or lower, they lose weight; but if they are kept at 90 per cent. either at 23 or at 30° C., they gain (Buxton, 1930*a*). The mean gain in weight has been shown to be water, the proportion of which rose during 23 days at 30° C. and 90 per cent. humidity from 57 to 65 per cent. of the total weight of the insects. In my original paper, it was suggested that the gain in weight might be due either to "hygroscopy" or to retention of metabolic water, but the second alternative is not possible. Fat is the only reserve substance which produces more than its weight of water, and even fat only produces a gain of 16 per cent.: the live meal-worm contains about 15 per cent. of fat, which would be quite insufficient to account for the gain observed. There is, then, no doubt that meal-worms can gain water from an atmosphere which is 90 per cent. saturated.

The data published by Bodine (1921) on the hibernating nymph of *Chortophaga viridifasciata* (Acridiidae) show that it has the same power of absorbing water from the air. In each of his experiments, he exposed five hibernating larvae in a closed vessel over wet sand. It is presumed that he excluded the possibility of water condensing on or near the insects, and being swallowed. The results that he obtained were as follows:

At 4° C.,	weight	lost	1·4 %,	water content rose	3·5 %,	48 hours
At 15° C.,	,,	gained	3·5 %,	,,	0·5 %,	48 ,,
At 23° C.,	,,	gained	14·3 %,	,,	22·6 %,	48 ,,
At 38° C.,	,,	gained	15·5 %,	,,	28·0 %,	24 ,,

Similar insects exposed to the same temperatures over calcium chloride lost weight and water at least as fast as these insects gained them.

The cyst of the Coccid bug, *Margarodes vitium*, which was referred to many years ago by Mayet (1895), is an example of a hygroscopic insect. At the end of its larval growth, this insect becomes completely covered with a waxy coating and it is then known as a "ground pearl." In this state it is able to resist prolonged drying, and doubtless it has to do so in nature, for it is found in soil in Chile and other arid parts of South America. When the cyst is put in damp soil, metamorphosis follows. One of Mayet's cysts produced an adult 6 years after the cysts had been collected, their age at the time of collection being unknown. More recently, Ferris (1919) has examined cysts of the same species which had been collected in Chile and kept in a museum in California for at least 17 years. Most of them were dead, but one contained a living insect.

Other facts suggest that many insects have this power, though the possibility that they drank water was not definitely excluded. Breitenbecher (1918) states that adult potato beetles (*Leptinotarsa*, Chrysomelidae) absorb moisture from the air when the relative humidity is high. Wigglesworth (1931 *b*) starved unfed bed-bugs in the first larval stage: as they used up their tissues and water, they maintained their general shape by swallowing air. If insects in this state are put into saturated air, some of them recover: it is certain that they gain water, and it is highly probable that they absorb atmospheric moisture, but it is possible that they drink minute drops which condense on the surface of the insect or on the container. Some species of Collembola probably have the same power. Davies (1928) dried insects of this order until they were moribund and visibly shrunken. He then put them on the surface of water and they swelled and became normal: this was presumably due to absorption, not drinking. The larvae of many gall-midges (Cecidomyidae, Dipt.) pass into a resting state before pupation. If conditions are dry some species can rest for a couple of years. When the surrounding soil is wetted the larvae swell up, and pupate: presumably they take in water through the skin (see Marchal, 1897; Enock, 1891; Barnes, 1927; see also von Gelei (1930) on the larva of a Chironomid midge, *Dasyhelea*).

I have (p. 300) quoted instances of hibernation coming to an end when insects take in water. Many of the insects which inhabit arid countries are dormant for a considerable part of the year: they awake when rain falls. We do not know whether these reactions are due to the insects drinking water in spite of their apparently dormant condition, or to their gaining water through their surface, which is more probable.

The facts are sufficiently clear: let us discuss the physiology of the process. It has been shown that if the possibility of drinking is excluded, certain insects gain water from a moist atmosphere, or from moist soil; this is demonstrated for a few insects and there is evidence which suggests that it may occur in many others. It is not easy to understand the mechanism which may produce this result. We have used the word "hygroscopic" loosely for these insects, but the phenomenon has little or nothing in common with the hygroscopy of hair, cotton and other dead substances. This is clear on several grounds: the ground pearl, and to a lesser extent the fasting meal-worm, are almost proof against loss of water, though they can gain it; the hibernating grasshopper can absorb water much more rapidly at a high temperature than a low. Furthermore, even if the insect possessed some hygroscopic substance, this would be of doubtful advantage, for the substance would hold the water and might even withdraw water from the insect.

At first sight a physical explanation seems possible. The insect has a vapour pressure, which is less than the saturation vapour pressure of water at the same temperature because of the presence of solutes in its fluids; water might therefore condense into it from a nearly saturated atmosphere. This may happen on the surface of the body, if the insect is one of those which lose water at this situation; or it might occur at the surface of the liquid which fills the terminations of the tracheoles. But on closer examination one concludes that this way of gaining water cannot be effective. If we assume a concentration of 1 per cent. sodium chloride in the liquid in the tips of the tracheoles (and one cannot assume high concentrations here, in the light of the facts given by Wigglesworth, 1930 and 1931 *c*), then the lowering of vapour pressure of water, at 18° C., is less than 0·01 mm. of mercury: but the saturation vapour pressure at this temperature is 15·5 mm. Condensation of water into the insect from an atmosphere 90 per cent. (or even 99 per cent.) saturated would therefore not occur. There is a second physical explanation which might be considered. Because the meniscus which separates liquid from air in the tracheoles is convex towards the liquid, condensation from the atmosphere into the insect will be favoured. But this also appears insufficient to account for the results observed. It seems that the facts point to the existence of a definite biological activity; it is possible that water is continuously secreted from the liquid in the tracheoles into the body of the insect; this would raise the concentration of the liquid and cause further water to condense into it. In favour of the view that the explanation is not purely physical, but that secretion of water into the insect is involved, is the fact that grasshoppers gain water more rapidly at a high than at a low temperature.

III. LOSS OF WATER.

(1) *Loss from alimentary canal.*

The insect may lose water with the contents of its alimentary canal, and also perhaps through the general surface of the body (including the surfaces at which respiration takes place). Water lost from the alimentary canal may be derived from Malpighian fluid, or liquid faeces. In many insects the loss from these two sources is considerable, not to say profuse, but many others conserve the water which would otherwise be lost through the anus. They do so partly by excreting uric acid, which is insoluble and therefore needs no water to take it away. They also possess very effective mechanisms for extracting water from the contents of the rectum: in certain insects this power is apparently exercised by the rectal epithelium in general, and in others it is localised to groups of cells which have generally been described as rectal glands. In these ways the loss of water through the alimentary canal is reduced to a very low figure by many insects: examples can be found in all the principal Orders both among larvae and adults. As Wigglesworth (1932) has recently published original work on this subject and also reviewed existing knowledge of it, there is no need to deal with the matter at length. But this type of water conservation is doubtless of very great importance.

But it would be useless for the insect to economise water in the manner dealt with above if it was rapidly losing it from the surface of the body. We know that many insects exercise economy of both types. The meal-worm (larva of *Tenebrio molitor*) is a good example: the cuticle is thick and waxy: in the rectum the contents of the hind-gut are dried with great efficiency: the Malpighian tubes excrete uric acid. As the insect can economise water in all these different ways, it is not surprising that it has remarkable powers of surviving desiccation. Individuals were starved at room temperature over strong sulphuric acid, and the first death occurred on the 210th day of the experiment (Buxton, 1930*a*). As additional examples we may quote Giard's (1896) observations that the larva of *Melanostoma* (Syrphidae, Dipt.), which normally lives in a very moist place, can be dried into a hard shrunken mass and kept in that condition for several weeks, without dying. Even more remarkable is the case quoted by von Gelei (1930): larvae of *Dasyhelea* (Chironomidae, Dipt.) which normally live at the bottom of ponds are capable of living many days in the mud if it is dried. The larva of *Hermetia chrysophila* (Stratiomyidae, Dipt.) is perhaps less remarkable because it lives on decaying cactus joints, and is liable to exposure to drought in its ordinary life; if the food becomes dry the larvae will live for at least a year and then start feeding again when it is moistened (Hunter, Pratt and Mitchell, 1912). Many almost equally remarkable examples of resistance to drying, and of dormancy under unfavourable conditions, could be quoted: several may be found elsewhere in this paper.

(2) *Anatomical site.*

We have little precise knowledge of the part of the insect from which water is lost, and most of the evidence is presumptive. We know that the finest tracheoles contain a liquid, the quantity of which is intimately related to the osmotic pressure of the blood; this liquid is present in the tracheoles of aquatic and also of terrestrial insects (Wigglesworth, 1931*c*). The gas which is in the rather larger tracheoles must be in equilibrium with the liquid which fills their terminations, and must therefore be close to saturation. It is a fair assumption from this that much of the insect's loss of water is by diffusion from the tracheal system through the spiracles. Hazelhoff (1926, 1927) has produced evidence that the spiracles are normally closed, so that loss of water through them is reduced. On the other hand, the rate of loss is doubtless increased, in certain insects, by respiratory movements. We may therefore regard it as certain that loss of water takes place from within the tracheal tree; to what extent do these animals also lose water from their general body surface? It appears that some of them lose none. The meal-worm (*Tenebrio*) is perhaps one; it loses water extremely slowly even in dry air at high temperatures, and the general surface of its body is waxy. The scorpion (*Opisthophthalmus*) is probably another example (see Zoond, 1931). Both the above animals—the meal-worm and the scorpion—are notoriously able to exist in dry places, and it seems that their loss of water is reduced to a minimum, and that it takes place only in respiration.

On the other hand, Buddenbrock and Rohr (1923) have shown that a quarter of the respiratory exchange of the stick insect *Dixippus* (Phasmidae) is through the integument, and that a considerable proportion of water is lost at the same place[1]. There is no doubt that this must be so, to an even greater extent, in certain minute Arthropods which have no tracheal system; *e.g.* certain mites, and many Collembola. With regard to the Collembola, it is to be remembered that some forms have tracheal systems, others not. The work of Davies (1928) has shown that several non-tracheate species die rapidly in dry air; for instance, at 25° C. and relative humidities of 30 per cent. or lower, every individual of *Isotoma viridis* was dead in half an hour, whereas in saturated air the first death took place in 2 hours and the last in 8 hours. But the species with spiracles and tracheae are more resistant to drying; *Sminthurus viridis* lives from 5 to 10 hours at a relative humidity of 0 per cent. If one may generalise from this, it appears that a tracheal system, combined with a relatively impermeable exterior, is advantageous, as it reduces loss of water.

(3) *Physical law of loss.*

The wheel diagram. The first attempt made to take a general view of the effect of different degrees of humidity at different temperatures was published by Pierce (1916). He plotted temperature and relative humidity on squared paper and laid down a number of concentric zones; the central zone included the combination of conditions which was optimal for the weevil, *Anthonomus grandis*. His figure has been often reproduced and his general method is frequently used by applied entomologists (see, for instance, Buxton, 1923*a*; Shelford, 1927; Bodenheimer, 1928 and 1929*a*, *b*; and others). The wheel diagram has been so much used that one must examine the method somewhat critically. Pierce's original diagram was drawn to cover all conditions of temperature from −19 to 82° C. combined with humidities from 0 to 100 per cent. But it appears that his experiments were only carried out under a few dozen combinations of temperature and relative humidity; more than half his diagram is occupied by concentric zones which run boldly across areas in which he has not recorded a single experiment.

Apart from this criticism of the original work, the method may be said to be convenient, though most of us would like to go deeper into the matter than the wheel diagram permits us to do: it is very cumbersome to say that an insect will breed at a particular relative humidity combined with a particular temperature, and one looks for something simpler and more physiological.

Law of saturation deficiency. So long ago as 1924, a paper was published by Bacot and Martin which points the way to a new conception[2]. At the time they wrote, it was well known that the relations between fleas and humidity were important, and Brooks (1917) had shown that there was a relation between the epidemiology of plague and the saturation deficiency[1] of the atmosphere. Bacot and Martin exposed adult *Xenopsylla cheopis* to four different humidities at 32° C. They were able to show that the duration of life of the insects, which were not fed during the experiment, was directly determined by saturation deficiency between the limits of 10 and 26 mm. of mercury; but the relation did not hold good for fleas kept in saturated air. They also showed that if the insects were exposed to the same saturation deficiency at two widely different temperatures, they lived longer at the lower temperature. It was only to be expected that the relation to saturation deficiency would break down in these circumstances.

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[1] It is known that liquid water and certain solutes can diffuse through thin layers of chitin (Gorka, 1914; Eidmann, 1922; Abbott, 1926), but this is not evidence that water vapour can do so. It might be argued that because the oxygen or carbon dioxide molecule can pass through chitin, water vapour can also do so. This argument is fallacious because it neglects the possibility of differential solubility.

[2] While this work was in the press Leeson brought out an important collection of fact relating to the duration of life of the same species of flea. It is clear that the duration is *not* proportional to saturation deficiency at any temperature, and that the phenomena are more complex than we have realised. The inconsistency between this work and that of Bacot and Martin cannot at present be explained.

It is a matter of considerable importance to decide whether Bacot and Martin's discovery can be extended to other insects, and if so, within what limits, and it is desirable that the problem should be attacked by direct methods: insects should be exposed to a slow stream of air of controlled temperature and humidity, and the water evaporated from them collected and weighed. Experiments of this sort have, so far as I know, never been carried out, but we have approached the problem indirectly in many ways. Several authors, apart from Bacot and Martin, have defined the period which is necessary to kill 50 or 100 per cent. of insects under defined and controlled conditions of temperature and humidity. Others have studied the loss of weight of certain inactive insects, it being shown or assumed that metabolism was low and that loss of weight could be regarded as approximately equal to loss of water. These methods are not only indirect but also crude, for the result may be affected by many things other than loss of water, particularly if the investigations are carried out over a wide range of temperature.

Attention may first be called to Kirkpatrick's (1923) large collection of facts obtained by exposing Lygaeid bugs (*Oxycarenus hyalinipennis*) to a great range of temperature and humidities. The work was done on hibernating adults, and the author used very large numbers of insects and presented his facts fully, so that others can make use of them. I have extracted from his figures the periods necessary to kill 50 per cent. of the insects[2]. It is, of course, possible to express his results by saying that half the insects die in 80 hours, either at 40° C. and 80 per cent. humidity or at 17° C. and 30 per cent. humidity: or that half the insects die in about 40 hours, either at 35° C. and 60 per cent. or at 30° C. and 40 per cent., or at 25° C. in dry air; that way of dealing with the facts is legitimate but cumbersome. If, on the other hand, one converts the humidity data to saturation deficiency (Buxton, 1931*b*), it is seen that with saturation deficiencies between 5 and 10 mm. of mercury, the mean duration of life is 80–167 hours: with saturation deficiencies over 30 mm., the duration is 5–18 hours, etc. It is not to be expected that the duration of life can be precisely related to saturation deficiency, or to any other measure of humidity; for the range of temperatures covered is great (10–45° C.). Within any particular limits of saturation deficiency, life is longer at lower temperatures than at higher, and no doubt the difference is due to the fact that death is caused partly by loss of water and partly by exhaustion of other substances.

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[1] Saturation deficiency is the amount of water vapour which would have to be added to a sample of air to saturate it, without altering the temperature: it is generally expressed as a vapour pressure in millimetres of mercury. The amount of water vapour which a given space will hold is greater, the higher the temperature: it will therefore require more water vapour to saturate air at 20° C. and 60 per cent. relative humidity than air at 10° C. and the same relative humidity. In other words, the relation between relative humidity and saturation deficiency is not simple, and one cannot convert readings from one scale to the other unless the temperature is known.

[2] Kirkpatrick's collection of facts is so large and covers so wide a range of temperature and humidity that it has been used by several authors. Bodenheimer (1928) and Weber (1930) have both discussed his facts, but rather in relation to vital optima than to the loss of water. Their graphs are discrepant in matters of detail, as Janisch (1931) has pointed out. Both authors made use of the periods necessary to kill all insects (not 50 per cent. of insects, which I have employed); but their graphs are consistent with my view that the duration of life is to a great extent determined by saturation deficiency, though they have not dealt with this point.

We can exclude one variable if we consider only the data collected at a single temperature, and we can then test whether Kirkpatrick's (1923) figures for the duration of life of *Oxycarenus* can be related to Dalton's law: according to this law, the loss of water evaporated is proportional to the saturation deficiency. If we take the mean duration of life at a particular saturation deficiency, and assume the validity of Dalton's law, we can calculate what the duration should be at any other saturation deficiency and the same temperature. In Table I, I present Kirkpatrick's figures for mean duration of life in hours and also the "expected" duration: this is based for each temperature separately on the duration at a relative humidity of 60 per cent. I chose this humidity as a starting-point because other insects do not follow the saturation deficiency law in saturated or in very dry air. The table shows that at 11° C. there is no agreement between the observed and the expected duration of life: but the original data are imperfect, for figures are only given by Kirkpatrick for the lower humidities. At 17, 25 and 30° C., there is a fair agreement between the observed and the expected figures. At 35, 40 and 45° C., there is also a fair agreement except when the relative humidity was over 80 per cent. In that group of experiments, the duration of life is much shorter than the expected duration. The explanation is perhaps that metabolism is rapid because of the high temperature, and that as evaporation is much reduced, the water balance of the insect is disturbed, causing death from excess of water, and not from its loss. This could readily be tested by determining the proportion of water in insects exposed to a number of combinations of temperature and humidity for different periods. On the whole, the agreement between observation and expectation is good, having regard to the fact that we are trying to relate a complex biological event to a simple physical law. Dalton's law probably does determine the loss of water from *Oxycarenus*, and therefore the duration of their life: but it cannot be applied to experiments in which a high temperature was combined with a high humidity.

Jones' (1930) data for the duration of life of the unfed first larva of the bed-bug (*Cimex lectularius*) and Grossman's (1930) for cotton-boll weevils (*Anthonomus grandis*) are consistent with the view that saturation deficiency is the effective measure of loss of water: but neither collection of data is large enough to furnish a decisive test.

Two authors have studied loss of weight or of water from insects which were quiescent. Bodine (1921) exposed hibernating nymphs of *Chortophaga viridifasciata*, a grasshopper, to various temperatures, keeping them in dry air at each temperature. In Fig. 1, I have plotted the loss of water per cent. from the insects against the saturation deficiency. It will be seen that the relation appears to be linear, at any

Table I. *Showing mean duration of life of* Oxycarenus *under controlled conditions of temperature and humidity, after Kirkpatrick. The table gives the "observed" duration, in hours; also the "expected" duration, which is calculated for each temperature separately, from the observed duration at* 60 *per cent. saturation, on the assumption that the duration of life is determined by Dalton's law.*

Temp. ° C.	Relative humidity %	Duration	
		Observed (hours)	Expected (hours)
11	1	82	68
11	20	138	85
11	40	168	113
11	**60**	**170**	**170**
17*	1	58	53
17*	10	60	58
17*	20	78	66
17*	30	82	75
17*	40	96	88
17*	50	106	106
17*	**60**	**132**	**132**
17*	70	194	198
17*	80	250	264
25	1	45	37
25	20	58	46
25	40	74	62
25	**60**	**92**	**92**
25	80	228	184
25	90	272	376
25	100	300	?
30	1	23	22·4
30	20	32	28
30	40	40	37
30	**60**	**56**	**56**
30	80	136	112
30	90	204	224
30	100	274	?
35	1	14	17·6
35	20	16	22
35	40	26	27
35	**60**	**44**	**44**
35	80	106	88
35	90	250(?)	176
35	100	158	?
40	1	8	7·2
40	20	11	9
40	40	13	12
40	**60**	**18**	**18**
40	80	82	36
40	90	62	72
40	100	56	?
45	1	5·0	4·12
45	20	6·5	5·15
45	40	8·5	6·9
45	50	9·1	8·2
45	**60**	**10·3**	**10·3**
45	70	16·4	13·7
45	80	17·4	20·4
45	90	6·3	40·8
45	100	5·0	?

* All experiments were at constant temperature except this group in which the temperature was between 15 and 19° C.

rate for saturation deficiencies of 6–20 mm.; this corresponds to dry air, from 4 to 23° C. This is remarkable when one notices the wide range of temperatures at which the insects had to be exposed in order to cover this range of saturation deficiency in dry air. My own work on the meal-worm (*Tenebrio* larva), which lies completely motionless if it has been starved for 2 days, showed that loss of weight was determined by saturation deficiency, but only within rather narrow limits of humidity. I understand from Mr K. Mellanby[1] that his unpublished data show that the relation of loss of weight in this insect to saturation deficiency is closer than appeared from my own work. Moreover, the metabolism of the fasting insect produces water which is almost equivalent in weight to the loss of material oxidised: in other words, loss of weight is a better measure of loss of water than we have previously realised (Buxton, 1930*a*).

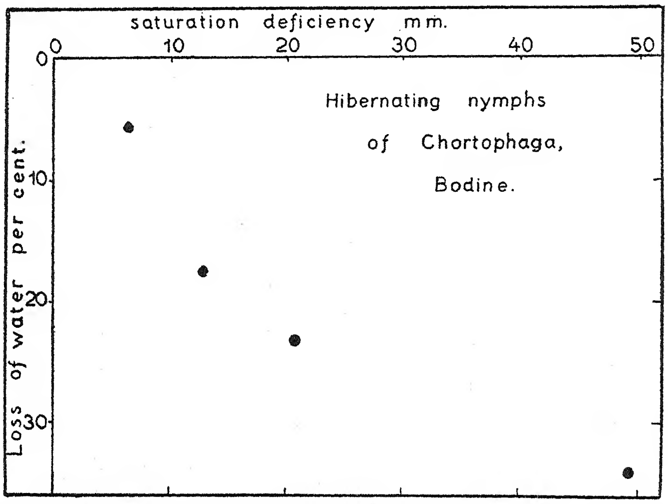

Fig. 1. Loss of water of hibernating nymphs of *Chortophaga*, subjected to various saturation deficiencies. (Data from Bodine.)

Limitation to the law. There is good ground for saying that the loss of water from an insect is proportional to saturation deficiency; but this is only the case within limits, which exist both at the dry and the wet end of the scale. Bacot and Martin (1924) showed that the duration of life of the flea was directly proportional to the dryness of the air, at a single temperature, when the saturation deficiency lay between 10 and 26 mm. of mercury. But when the insects were exposed in saturated air, this relation broke down: the insects were losing no water by evaporation, and died from some other cause. As we have already pointed out, the life of *Oxycarenus* is shortened by exposure to hot moist air. Something similar has been demonstrated by experiments on the eggs of the grasshoppers, *Melanoplus* and *Schistocerca* (p. 307). An exception, in hot dry air, was discovered in the course of work on *Tenebrio* (Buxton, 1930*a*). I showed that in dry air, the insect's weight fell about 30 per cent. during an exposure of 28 days. This happened both at 23 and also at 30° C., though the saturation deficiencies were 21 and 32 mm. of mercury: there is perhaps a maximum rate of loss of water from this insect.

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[1] See Mellanby (1932 *b*).

Exception to the law. Apart from these limitations, there are several collections of data which appear to be irreconcilable with the view that the saturation deficiency of the air determines the rate of loss of water from an insect. Davies (1928) has studied the duration of life of several species of Collembola at 25° C. His technique was good and his data are presented fully. It is clear that the duration of life of these insects is very much greater in moist air than in dry, and that the facts are irreconcilable with Dalton's law. But apart from any question of water loss, most of these insects die very rapidly from starvation; for instance, *Isotoma viridis* dies in 8 hours even if it is kept on a surface of wet cloth; and it is possible that the readiness with which they die from starvation makes them unsuitable for my present purpose. The work of Payne (1929) should perhaps be mentioned, though she was not considering the subject of water loss. In investigating certain insects which can resist low temperatures, she found that the temperature which the insects could withstand was directly related to the absolute humidity of the air to which they had previously been exposed; this was true also of an egg. This is an interesting and unexpected result, but her facts appear to be capable of no other interpretation.

On the evidence which has been given above, we are justified in saying that the saturation deficiency of the air directly determines the duration of life of fasting insects of several species. Loss of weight of two unrelated fasting insects is also directly proportional to the saturation deficiency of the air. These generalisations are only true at one temperature: if results obtained at several temperatures are considered, the increased metabolism which takes place at the higher temperature is generally enough to obscure any relation there may be to the saturation deficiency. The relation to saturation deficiency breaks down in very dry air, and in saturated air, and there are several collections of fact which appear to be irreconcilable with the law of saturation deficiency.

But even this is an important advance. It makes it possible to compare the climate of two spots, and to say that so far as an insect is concerned they are equally moist; or that the air in one would remove water from an insect three times as fast as the air in the other. It also enables a worker to expose insects to several different temperatures, the rate of loss of water being the same in each.

But if we accept the view that saturation deficiency gives the simplest and truest expression of the loss of water from an insect, we must cease to make use of relative humidity. There is no justification for halting between two opinions; if loss of water at two temperatures is proportional to saturation deficiency, it cannot be proportional to relative humidity.

(4) *Other studies on loss of water.*

The facts on which we may be able to formulate a law of loss of water from an insect have been given above. There are also collections of data relating to other effects of atmospheric humidity, for instance the temperature which is lethal, time and atmospheric humidity being defined; the insect's power of regulating its internal temperature by evaporation; and the duration of life under controlled conditions of temperature and humidity.

Thermal death-points. The upper lethal temperature of the newly hatched unfed larva of *Rhodnius prolixus* (Reduviidae, Rhynch.) is unaffected by the humidity of the air (Buxton, 1931 *a*). Even if it is exposed for 24 hours, the insect dies at 40° C. at relative humidities ranging from 0 to 90 per cent. This is remarkable, for it is slender and minute (0·5 mg.), so that the ratio of surface to volume is very great. Miller (1930) has shown the same thing with pupae of *Epilachna corrupta*. Mellanby (1932) shows that small meal-worms (*Tenebrio* larvae), adults and larvae of fleas (*Xenopsylla cheopis*), and adults of *Pediculus humanus* (Anoplura) and *Lucilia sericata* (Muscidae, Dipt.) die at a definite temperature[1], which is not affected by the humidity of the air, provided the exposure is for 1 hour. But if they are exposed to dry air for 24 hours, the flea larva, the *Pediculus* and the *Lucilia* lose water, so that their death-point is many degrees lower at a relative humidity of 0 per cent. than it is in air which is 90 per cent. saturated. The flea larva shows this to an extreme degree. At 90 per cent. humidity it dies at 36° C.; at 60 per cent., 32° C.; at 30 per cent., 27° C.; at 0 per cent., 22° C. In other words, the flea larva (like the Collembola, see Davies, 1928) dies of desiccation: this may in part be due to its restlessness, and to loss of water through its chitin; but it must in part be due to the fact that its rectal epithelium does not dry its faeces very efficiently (Wigglesworth, 1932).

In contrast to this, the cockroach (*Blatta*) survives best in drier air; it dies at 38° C. in moist air, 48° C. in dry air (Bodenheimer, 1927). This ability to survive a short period in drier air is doubtless due to the insect cooling itself by evaporation (see Necheles, 1924).

The facts regarding the adult, or the larva, of *Epilachna corrupta* are more complex (Miller, 1930). Miller's figures for adults, exposed to various conditions for 3 hours, are plotted in Fig. 2. It will be seen that at least 90 per cent. of the adults were killed by saturated air at 39·5° C., or dry air at 40·5° C.; but at humidities above 60 per cent. some survived 40·5 or even 41·5° C. The larvae behave in a somewhat similar manner. Beattie's (1928) observations on adult *Calliphora erythrocephala* (Muscidae, Dipt.) are essentially similar. Similar facts are available for other insects, though these have not been so completely studied. (See Mehta, 1930, for *Dysdercus*; Goodwin, 1914, 1922, for beetles in grain.)

It is clear that if insects are exposed to a range of humidities at a temperature which is lethal or nearly so, one of several things may happen. Certain insects are unaffected by the humidity, even with a 24 hours' exposure (*Rhodnius*). Others, like the flea larva, die at a lower temperature in dry air, by loss of water. Others again, for instance the cockroach, can survive a higher temperature in dry air, and this is presumably owing to evaporation of water from the insect's body; the observations of Necheles require to be confirmed and extended. There is yet another group which survive best at moderate humidities: presumably under these conditions the insects can evaporate enough water to cool themselves, and not enough to cause fatal loss of water: adult *Calliphora* and *Epilachna* (Fig. 2) are examples. But the power of regulating internal temperature by evaporation is limited by the amount of water which the insect can lose without disturbing its internal economy. Mellanby (1932) brings forward reasons for believing that regulation of temperature can only occur in relatively large insects. In small insects, in which the surface is great with reference to the volume, the gain of heat by convection must be greater than could be balanced by evaporation of water even during so short an exposure as an hour.

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[1] One would be justified in speaking of the lethal temperature in saturated air as the "true lethal temperature," because under these conditions we have death determined by temperature, without any complication caused by evaporation.

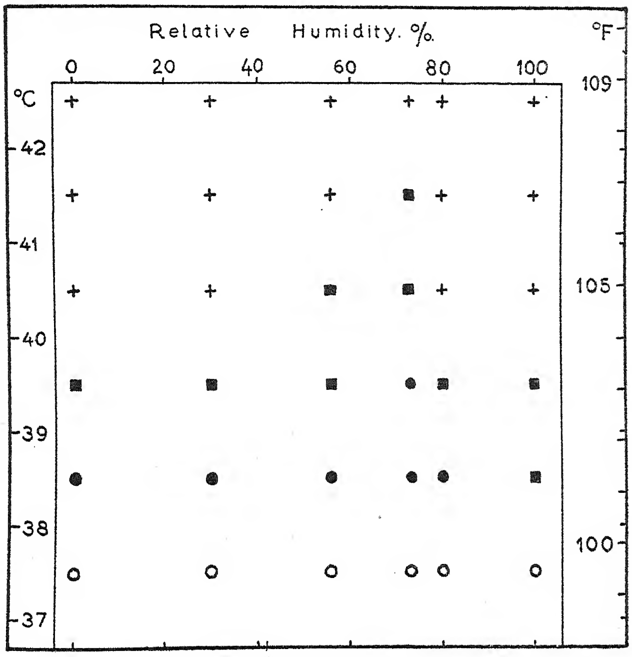

Fig. 2. Thermal death-point of *Epilachna corrupta* after an exposure of 3 hours to controlled temperature and humidity. White circles denote no deaths; black circles, deaths up to 50 per cent.; black squares, deaths over 50 per cent.; crosses, deaths 95 per cent. or over; a few controls died. (Data from Miller.)

Regulation of internal temperature. From the foregoing paragraphs it is clear that the insect regulates its internal temperature, when the external air is hot and dry. But it also possesses powers of regulation when the external air is much below the lethal temperature. Bachmetjew (1901) impaled a hawk-moth, *Deilephila euphorbiae* (Sphingidae, Lep.) on a thermocouple, and subjected it to rising temperature in air which was not artificially moistened (the saturation deficiency presumably increased with rising temperature). At the start of the experiment at 37° C., the temperature of the insect was 0·3° C. below that of the air; the increase became greater, and when the chamber was at 47·6° the insect's temperature was 2·5° C. lower. Beyond this point the two temperatures tended to become closer, and they were identical at 51·5° C. When a related insect, *D. elpenor*, was exposed to rising temperatures in a moist thermostat, the insect's temperature was above that of the surrounding air. The results of the first experiment carried out in unmoistened air might perhaps be due to a delay in the penetration of heat through the insect's body, but taken in conjunction with those in moist air, it seems established that these moths regulate their temperature by evaporation. The work of Necheles (1924) on cockroaches leads to a similar conclusion; lowering of temperature is not efficient above 40° C., perhaps because the insect is receiving more heat by conduction than it can pass off by evaporation, perhaps because the available water in the insect has been nearly exhausted earlier in the experiment. Locusts (*Schistocerca gregaria*) possess a similar power of lowering the body temperature if they are placed in dry air at about 40° C. (Bodenheimer, 1929*a*). Specimens of *Scarites eurytus* (Carabidae, Col.) behaved similarly, and the rise of temperature of dead and live individuals was at the same rate (Bodenheimer and Samburski, 1930). These authors say that no loss of weight could be detected before and after exposure to moist or dry air: they appear to hold the view that the differences observed in the temperature of insects exposed in moist and dry air can be explained without recourse to evaporation.

Regulation of the insect's internal temperature has also been shown to occur in deserts, in summer. Under these conditions, the insect is living on a very hot surface, and is itself receiving solar radiant heat. The internal temperature of certain beetles and grasshoppers was shown to be much below that of the surface on which they rested, though much above shade temperature (Buxton, 1924*a*). In this case, the live insects were several degrees cooler than those freshly killed, which shows that the facts cannot be due to lag; it is possible that their respiratory movements enable the live ones to evaporate water through the tracheal system.

Duration of life[1]. The duration of the life of insects under defined conditions of relative humidity is of interest in relation to Bělehrádek's (1926, 1930) view that the speed of the vital processes is largely determined by the viscosity of the protoplasm. So far as insects are concerned, it appears that Bělehrádek has only considered the early work of Hennings (1907 *a*, *b*). He dealt with *Ips* (*Tomicus*) *typographus*, a small beetle which lives in galleries under bark throughout its early stages and for a part of its adult life. He had several large thermostats, each divided into two compartments. In one, the air was kept nearly saturated; in the other, it was dried with calcium chloride, but owing to leakage and moisture from the experimental material, the relative humidity in this compartment was about 55 per cent. In each compartment he put twigs containing beetles in various stages: needless to say, it was not possible to measure or control the humidity in the galleries under the bark of the twigs. Hennings tabulates the duration of the successive stages of the insect, but it is not easy to understand how he established his facts as nearly all the events were taking place in the burrows. The data on the duration of larval life must be disregarded, because the differences that he observed in the dry and wet compartments may have been directly due to atmospheric humidity, or to its effects on the wood which the larvae were eating. But his data on the duration of the pupal stage are not open to this objection. The stage occupied a longer period in dry air (55 per cent. relative humidity) than in moist air, and this occurred at all the four temperatures available.

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[1] See also p. 285.

There is little other work that can be quoted. Headlee (1917) exposed pupae of the moth *Sitotroga cerealella* (Tineina) and the beetle *Bruchus obtectus* to a range of relative humidities at 27° C. The duration of the pupal stage in days was found to be as follows:

Insect	Relative humidity %			
	100	73	45	22
Sitotroga	17	17	16	12
Bruchus	22	21	14	15

Ten or twenty individuals were exposed to each humidity, but the author does not state whether his results were consistent. Taking the available facts, it appears certain that the effect of lower humidity is the opposite to that observed by Hennings. According to Holdaway (1928), the duration of the pupal stage of *Tribolium confusum* is unaffected by humidity between 0 per cent. and saturation at 27° C. There is also inconclusive work by Hefley (1926, 1928) and Melvin (1931). It will be seen from the facts collected above that the hypothesis of Bělehrádek receives no real support. Even if Hennings' data could be accepted, and if other data were consistent, no positive evidence about viscosity would be available. We shall refer to Bělehrádek's view again when we discuss the effect of humidity on the duration of the egg stage in insects (p. 313).

Apart from the above facts, there exists a considerable collection of data relating to the duration of life of a number of adult insects, most of them unfed. The facts, in spite of their economic value, appear to have little theoretical interest. Robinson (1926*b*), Headlee (1917), Gill (1921*a*), Bodenheimer (1929*a*) and Titschak (1926) have dealt with insects of several orders, which lived longer when they were starved in moist[1] air than in dry. Facts of a similar nature are recorded elsewhere in the present work; see, for instance, Bacot and Martin (1924), Davies (1928), Kirkpatrick (1923), and Roubaud (1909).

Several authors have also studied the duration of life of particular stages of insects, maintaining them on their natural food plant and supposing that a control of humidity was achieved (see Sweetman and Fernald, 1930; Grossman, 1930; Headlee, 1914, 1921; Macgill, 1931; Weed, 1927).

IV. WATER BALANCE.

I propose to state what we know of the normal proportion of water in insects of different kinds, and then to examine the manner in which it is maintained, and the changes which may be induced by season and by growth and metamorphosis.

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[1] Though starved insects generally live longer at a high humidity, saturated air particularly at high temperatures is unfavourable—see p. 297.

(1) *Proportion of water in insects.*

In Table II, I have gathered together all the records that I can find of the percentage of water in what might be called normal insects. I have excluded data relating to insects exposed to unusual temperatures, or preparing for hibernation; these and other topics are discussed later. The percentages in the table must not be critically compared with one another; if they are to be used, the original papers should be consulted. The facts were collected by different workers who nourished and treated their insects in different ways, and employed different methods for estimating the proportion of water. Vinogradov (1929) has recently pointed out that the ordinary methods of desiccation cause the insect to lose considerable quantities of volatile substances other than water. It is also to be remembered that, at any rate in some insects, the proportion of water differs greatly in individuals reared under identical conditions.

The following particular points in Table II call for comment. The larvae of *Rhodnius prolixus* were unfed in the first stage. The inconsistent data relating to *Galleria* larvae may be due to differences in size: Teissier's (1931) data are the most complete and convincing. The data for meal-worms (larvae of *Tenebrio molitor*) are very inconsistent. Hall (1922) records 49·8 per cent. of water, and says that his figure is an average based on sixty insects dried at 99° C. Berger (1907) found 65 per cent. of water in small larvae; 61·6 per cent. in larger individuals. Teissier showed a similar increase in solids, with rising weight, and studied it very fully (see p. 300). Taking my data (Buxton, 1930*a*) for thirty-six individuals weighing from 80 to 140 mg., the mean percentage of water was 57·132, but the range was from 50·5 to 66 per cent. An inconsistency may be noted between two determinations by Robinson (1926*b*, 1928*c*) of the proportion of water in adult rice weevils (*Calandra* (*Sitophilus*) *oryzae*): the differences are apparently due to methods used in estimation. Sweetman's percentage of water in the larva of *Lachnosterna* (*Phyllophaga*) is stated

Table II. *Showing the percentage of water in a number of different insects.*

Family	Name	Stage	% water	Author
		ORTHOPTERA.		
Blattidae	*Periplaneta americana*	A	70·4–74·8	Gunn
,,	*Blatella germanica*	A ♂	68·4	Buxton, MS.
,,	,,	A ♀	66·6	,,
Acridiidae	*Melanoplus femur-rubrum*	L	77·6	Bodine, 1921
,,	,,	A ♂	74	,,
,,	,,	A ♀	72·6	,,
,,	*Melanoplus differentialis*	A ♂	69·4	,,
,,	,,	A ♀	68·0	,,
,,	*Dichromorpha viridis*	A ♂	67·3	,,
,,	,,	A ♀	70·8	,,
,,	*Chortophaga*	L	73–76	,,
,,	,,	A	61·5–67	,,
		RHYNCHOTA.		
Reduviidae	*Rhodnius prolixus*	L	74	Buxton, MS.
,,	,,	A ♂	70·6	,,
,,	,,	A ♀	70·6	,,
,,	*Triatoma rubrofasciata*	A ♀	68·4	,,
Cimicidae	*Cimex lectularius*	A	69·5	,,

Table II (*continued*).

Family	Name	Stage	% water	Author
		Lepidoptera.		
Tineidae	*Tinea pellionella*	L	57·6–59·8	Babcock, S. M.
Pyralidae	*Ephestia kuehniella*	L	64·2	,,
,,	,,	P	65·5	Speicher
,,	*Galleria mellonella*	L	64	Sieber and Metalnikow
,,	,,	L	74–55	Teissier
Nymphalidae	*Vanessa antiopa*	L	77–79	Robinson, 1928*c*
Pieridae	*Pieris rapae*	L	83–84	,,
Saturniidae	*Callosamia promethea*	P	70	,,
,,	*Telea polyphemus*	L	90–92	,,
,,	,,	P	71–75	,, 1927, 1928*c*
Bombycidae	*Bombyx mori*	Egg	64–69	Farkas
,,	,,	L	77–79	,,
,,	,,	P	76–79	,,
,,	,,	A ♂	70	,,
,,	,,	A ♀	79	,,
Lymantriidae	*Euproctis chrysorrhea*	L	82·9	Sacharov
Noctuidae	*Chorizagrotis auxiliaris*	L	83–88	Robinson, 1928*c*
,,	*Cirphis unipuncta*	L	87–89	,,
,,	*Euxoa segetum*	L	84·7	Sacharov
,,	,,	Lh	71·4–75·6	,,
,,	*Scoliopteryx libatrix*	Ah	48–65	,,
		Coleoptera.		
Tenebrionidae	*Tenebrio molitor*	L	49·8	Hall
,,	,,	L	61·6–65·0	Berger
,,	,,	L	57	Buxton, 1930*a*
,,	,,	L	58·5–59·5	Schulz
,,	,,	L	64·0–55·1	Teissier
Bruchidae	*Bruchus obtectus*	A	47·8–51·3	Babcock, S.M.
Chrysomelidae	*Leptinotarsa decemlineata*	A	80	Breitenbecher
,,	,,	A	62–66	Robinson, 1928*c*
,,	,,	A	76·4	Fink
Cerambycidae	*Cyllene robiniae*	A	56–60	Robinson, 1928*c*
Curculionidae	*Calandra oryzae*	A	64·8	Robinson, 1926*b*
,,	,,	A	48–50	Robinson, 1928*c*
,,	*Calandra granarius*	A	46–47	,,
Scarabaeidae	*Lachnosterna* sp.	L	73–82	,,
,,	,,	L	77·7	Sweetman, 1931*a*
,,	*Lachnosterna implicata*	Ah	77	,,
,,	*Melolontha hippocastani*	Lh	79·2	Sacharov
		Hymenoptera.		
Apidae	*Apis mellifica*	A	74	Sacharov
Tenthredinidae	*Cimbex americana*	L	78–82	Robinson, 1928*c*
		Diptera.		
Culicidae	*Culex pipiens*	Ah	56–58	Buxton MS.
Muscidae	*Calliphora vomitoria*	P	67·1	Weinland
,,	,,	A	68·4	,,
Oestridae	*Gastrophilus equi*	L	62–78	Kemnitz
Muscidae	*Ophyra cadaverina*	L	69	Tangl
,,	,,	P	59–53	,,
,,	,,	A	66·6	,,
		Siphonaptera.		
Pulicidae	*Xenopsylla cheopis*	A	80	Bacot and Martin
		Arachnida, Acarina.		
Ixodidae	*Ornithodorus moubata*	A	69·7	Buxton MS.

L = larva; P = pupa; A = adult; h = hibernating.

to be "free water." The percentage of water in *Xenopsylla cheopis* given by Bacot and Martin (1924) must, I think, be accepted with hesitation. It appears that they obtained their percentage by relating the final dry weight of fleas which had been starved to death to the original wet weight of the insects: they took no account of loss of solid material owing to metabolism.

Data on the proportion of the total water which is "bound" or "free" are referred to on p. 302 below.

(2) *Optimum humidity*.

The work of applied entomologists and ecologists contains many examples of insects which are closely dependent on particular conditions of atmospheric humidity. Among insects which require a high degree of humidity one might mention the Mexican bean beetle (*Epilachna corrupta*, Coccinellidae, Col.) (see Sweetman, 1929; Sweetman and Fernald, 1930; Symposium, 1931; Marcovitch and Stanley, 1930). Another example is the tsetse fly (*Glossina* spp.; Muscidae, Dipt.) (see Newstead *et al.* (1924) for a full general account of biology; also Swynnerton, 1929; Nash, 1931; Lester and Lloyd, 1928). There are many insects which normally live in a very dry environment. There is a mass of information on insects which infest stored products; and on the biology of fleas, the early stages of which live in apparently dry organic debris. I have given a general account of biological conditions in deserts (Buxton, 1923*b*; see also Wheeler, 1931).

It is simple though tedious to delimit the conditions of temperature and humidity which are fatal to an insect (p. 290), but it is much more difficult to discover those which are optimal: indeed, it is frequently impossible, particularly with insects which are feeding: but it is possible for an insect which is not feeding, whether it be a pupa, an egg, or a resting larva or adult. It follows from our general knowledge of ecology that different stages of the same insect may have widely different optima; I know no grounds for Bodenheimer's (1927) suggestion that insects are generally less sensitive to low humidity than to high, nor for Bachmetjew's (1901) belief that insects containing a high proportion of water would be more readily killed in hot dry air than those with a lower proportion.

Several examples can be found in the literature of insects which are unfavourably affected by moist air. Bataillon (1893), for instance, carried out experiments many years ago on silk-worms, particularly at the time of metamorphosis. He found that loss of water was very much reduced if the insects were kept in moist air: presumably, therefore, active elimination of water by the Malpighian tubes was not taking place and the insects relied on evaporation. Successful pupation was delayed, or impossible, for larvae kept in moist air. Cunliffe's (1921, 1922) experiments on the effect of dry and moist air on the ticks *Ornithodorus moubata* and *savignyi* (Ixodidae, Acarina) gave him the following results:

Material	With excess of water	With trace of water daily	With calcium chloride
O. moubata, 50 eggs in each chamber	No adults: one reached 4th instar	8 adults	33 adults
O. savignyi, 33 1st stage nymphs in each chamber	3 adults	9 adults	15 adults

Here again there is clear evidence that moisture was unfavourable. In this connection, one may remember that *Ornithodorus moubata* is widely distributed in Africa wherever the climate is dry, but that it avoids the rain forests. Its distribution has been mapped by Becquaert (1930). Readio's (1931) experiments with the hibernating larvae of *Reduvius personatus* (Reduviidae, Rhynch.) seem to show that these insects also have a low optimum humidity.

In contrast with the silk-worms and the ticks, there are many insects which are only able to exist in moist air. Elwyn (1917) has studied the effect of different degrees of atmospheric humidity on the pupa of *Drosophila ampelophila* at about 18° C. His results are as follows:

At a relative humidity of	100 %,	401 pupae,	97·5 %	hatched
,, ,, of about	65 %,	333 ,,	87·7 %	,,
,, ,, of	0 %,	403 ,,	47·0 %	,,

He showed also that the mortality at the lower relative humidities was still higher in pupae which were under 8 hours old. Melvin (1931) has clearly shown that the newly formed pupa of *Stomoxys* is readily killed, even in an atmosphere 75 per cent. saturated, at 25° C. There are a number of papers on the relation between subterranean insects and the water content of soil. They are difficult to interpret because the effect of the water may be directly on the insect, or due to mechanical differences between wet and dry soil. (See Sweetman (1931 *a*) on larvae of chafers (*Lachnosterna*); Parker (1915) on *Pemphigus*, an Aphid attacking roots of beet; Wardle (1930) on 3rd stage larva of *Lucilia* (Muscidae).) Many other examples could be quoted of conditions of humidity which are known to be favourable or unfavourable to certain stages of insects. Some of them can be found elsewhere in the present paper[1].

Even those insects which require a high degree of humidity are not generally favoured by saturation, particularly if it is accompanied by high temperatures. This is clearly shown in Zwölfer's (1931) work on *Panolis flammea* (Noctuidae, Lep.). The insect hibernates as a pupa, and produces one generation annually, the larva eating pine needles. If the insects are kept in saturated air, many of their vital processes are disturbed. The total production of eggs is reduced in females kept in saturated air, as compared with those kept at a relative humidity of 90 per cent. Something similar is shown by the number of eggs actually laid. In a series of experiments extending from 8 to 27° C., females in saturated air laid less than 20 eggs, and generally less than 10. In air with a relative humidity of 80–90 per cent., they laid 10 eggs at 8° C., rising to 105 at 18° C. and falling to 24 at 27° C. The unfavourable effect of saturated air is also shown by the fact that in it less than 20 per cent. of females copulate; this occurred at a number of temperatures from 8 to 22° C.:

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[1] Inasmuch as there are optimal climatic conditions for insects, it follows that outbreaks of such diseases as malaria and plague are partly determined by seasonal alterations in the birth and death-rates of the insect vectors. It was suggested by Bentley (1911) that "there is the further possibility that atmospheric humidity may influence the development of the malaria parasite in the body of the mosquito." This possibility remains unproven; but the work of Mayne (1928, 1930) supports the view that in dry air mosquitoes do not transmit *Plasmodium* or *Proteosoma*, partly because their life is short, but partly also because of some effect of the humidity on the internal economy of the insect: see also Gill (1921 *b*, *c*). Rao and Iyengar (1930) are inclined to believe that *Culex* cannot develop the early stages of the Nematode worm, *Filaria bancrofti*, in dry air, but their data are not conclusive.

the normal percentage was 50–80. Furthermore, the average duration of life at five different temperatures is less in saturated air than at a relative humidity of 80–90 per cent.; this is true of each sex separately, and the differences are clearly significant.

Other examples of insects unfavourably affected by saturated air are known. *Epilachna corrupta* (Chrysomelidae, Col.) is intolerant of saturation at 32° C. (Sweetman and Fernald, 1930). Nymphs of *Sminthurus* (Colembola) live longer at 70–90 per cent. humidity than in saturated air (Maclagan, 1932). Compare also *Oxycarenus* (p. 286); pupa of *Tribolium confusum* (Holdaway, 1928) and certain eggs (p. 309).

The instances quoted show that air which is almost saturated with moisture is unfavourable to many insects, particularly at high temperatures: the insects belong to several different orders; the effect is observed both on early stages and adults; and (in *Panolis*) the effect is demonstrated in a number of different ways. This "water poisoning" is probably due to several different causes; the insect cannot lose water by evaporation if the air is saturated; it may be gaining water which condenses into it from the atmosphere; particularly at high temperatures it is producing a considerable quantity of water of metabolism. It would be of value to estimate the proportion of water in insects after exposure to air of various degrees of humidity: this has been done for *Tenebrio* larvae but for none of the other insects mentioned.

(3) *Power of regulating water content.*

Certain insects possess the power of regulating the proportion of water in their bodies. This has been studied in the meal-worm, the larva of *Tenebrio molitor*. At 23° C. the proportion between dry matter and water is kept constant in the meal-worm, even if batches of insects are starved at several relative humidities for a month (Fig. 3). The accuracy of regulation is remarkable, for a month over strong sulphuric acid, which brings the insect's weight down to 68 per cent., only raises the proportion of dry matter from 42·2 to 44·0 per cent.; this stabilisation of water content is carried out by solids, and by making use of the resulting water of metabolism (Berger, 1907; Buxton, 1930*a*). But the loss of water from this insect is proportional to the saturation deficiency. From this it follows that the rate of metabolism is determined by the atmospheric humidity: this is proved for the fasting meal-worm and may, I think, be presumed to hold good when it is feeding; this adds to the considerable list of factors which influence the rate of metabolism of an insect. Sweetman's (1931*a*) work on the adults of *Lachnosterna* (*Phyllophaga*) *implicata* shows a somewhat similar power of regulation. Wigglesworth's investigations (1931*c*) have brought him to the same conclusion. He has studied the extent of air in the tracheoles of various terrestrial insects, particularly a species of flea: he shows that the extent of the air is a measure of the osmotic pressure of the body fluids, and that this is preserved unaltered till after death if the insect is starved. Speicher bred *Ephestia* in dry air, and in the ordinary air of the room: the pupae from the culture in dry air weighed less than half the normal, but contained the normal proportion of water.

There remains the alternative possibility, that certain insects can tolerate great loss of water from the tissues. In this connection the observations of Giard (1902) are suggestive. He found larvae of *Sciara medullaris* (Mycetophilidae, Dipt.) living in the pith in stems of the ragwort (*Senechio jacobaea*). When he exposed larvae to the air of a warm room, they became dry, motionless and opalescent. But when he transferred the stem, which had been dry for 3 weeks, to a damp chamber, the larvae became active and shiny in a few hours. It is possible that these larvae approach the eutectic condition, and that they can resist high temperatures if they are able to lose water from their tissues. The only insect on which this has yet been shown is *Leptinotarsa*; normal individuals die at 58–60° C., desiccated ones 1–5° higher, the period not being stated (Breitenbecher, 1918, p. 378).

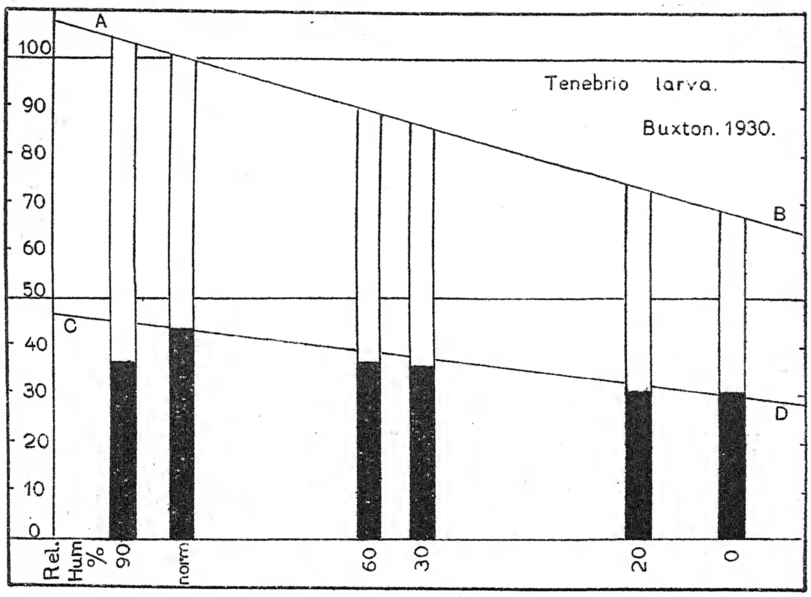

Fig. 3. Proportion of water and dry matter in meal-worms (larvae of *Tenebrio*); black is dry matter, white is water. The lines *AB*, *CD* and the base line all meet at a point: inasmuch as the line *CD* almost exactly cuts the junction between black and white in the columns which correspond to larvae starved at 60, 30, 20 and 0 per cent. humidity, it is clear that the loss of weight was not accompanied by any disturbance of the ratio between dry material and water. Batches starved at 23° C., and various humidities, are compared with the control. (Data from Buxton, 1930*a*.)

In the preceding paragraphs, we have assumed that the optimum humidity is that at which growth, metamorphosis, etc., proceed with the least number of deaths. But one may think of the optimum humidity as that degree of atmospheric humidity in which the insect neither gains nor loses water. It should be easy to define this degree of humidity for a dormant insect, for instance the fasting meal-worm or the hibernating nymph of the grasshopper, *Chortophaga*: both of these are known to gain weight in moist air and lose it in dry air (p. 280). Bodenheimer's adult locusts, which only lose 0·8 per cent. of their weight per day, in saturated air, must be very nearly in equilibrium with it (Bodenheimer, 1929*a*, p. 512).

(4) *Alterations in water content.*

But though some insects possess remarkable powers of regulating the proportion of water in their bodies, the proportion may be different at different times of their life history. A few facts are also available relating to the proportion of water in insects of the same species but of different size, and also relating to the water balance that accompanies metamorphosis. With regard to seasonal changes, more is known, especially in relation to insects which inhabit cold climates and hibernate for a part of the year.

Growth. It is a general principle that the larger the organism, the greater the proportion of skeleton, for mechanical reasons. The proportion of water in the organism as a whole must therefore fall with increasing size. But this has no real relation to the problem of water balance, and though the proportion of water falls in the larger individuals, it is probable that it remains constant in the active organs. Facts relating to the proportion of water in insects of different sizes have been published by Teissier (1931) for larvae of *Tenebrio* and *Galleria*: every time the meal-worm doubles its weight its dry material is increased 2·085 times, its water 1·965 times. See also Kemnitz (1916) for larvae of *Gastrophilus equi*.

Bodine's (1921) studies on the proportion of water in grasshoppers show a similar state of affairs. For instance, the larva of *Melanoplus femur-rubrum* (Acrididae) contains 77·6 per cent. of water, the adult about 72 per cent. If adults are considered, the water content falls consistently with rising weight. This I should attribute to the mechanical reason which I have mentioned above, but the author himself thinks that the heavier individuals are the older and that the water content falls with rising age. I have similar data (unpublished) relating to *Rhodnius prolixus*: in this insect the proportion of water varies inversely with the weight, if one considers only insects of the same age and sex.

Metamorphosis. Changes in the water balance in relation to metamorphosis have been studied in several Muscid flies (*Calliphora vomitoria* by Weinland (1906); *Ophyra cadaverina* by Tangl (1909)); also in the silk-worm (*Bombyx mori*, Lep.) by Farkas (1903). It appears to be inadvisable to summarise their work here, for alterations in water content are only a trivial part of the great metabolic changes which accompany metamorphosis. According to Teissier (1931), the act of moulting from one larval instar to another does not alter the proportion of water in the meal-worm.

Dormancy. The relation between the water content of insects and their ability to hibernate is of vital importance. It has been studied in the potato beetle (*Leptinotarsa decemlineata* and related species). In *L. decemlineata* dormancy occurs in winter, but some of the species are dormant in summer. Tower (1906) found that the adults of *L. decemlineata* which emerged in September or early October ate readily and stored up a reserve of fat. They then ceased feeding, emptied their alimentary canals and eliminated fluid through their Malpighian tubes. This process took about 3–10 days, and during it the body weight fell 30 per cent.: nearly all this loss of weight was attributed to Malpighian activity, and the dry weight of the insects did not fall. The insects then burrowed into the soil and remained there for the winter. Fink (1925) made similar observations on *L. decemlineata* and added that the proportion of water in the insect, which had fallen in the period before hibernation, remained low during the winter. In the spring, but before the insect emerges from the soil and before it feeds, the proportion of water rises. Breitenbecher (1918) carried our knowledge of the insects of this genus further. He found that if he took adults in early October, that is to say before they were ready to hibernate, and buried them, they died; but if he desiccated and then buried them, as many as 90 per cent. survived until the following May. He also showed, as Tower had previously done, that dormancy could be prolonged for 12 months or more if the conditions remained suitable, and he says that insects sometimes remain dormant in nature for more than one year. He found that successful survival through the winter was dependent upon the insect being allowed to burrow in "adobe," by which we may understand a clay soil. If they were buried in coarse sand, they died, presumably because they dried up in this material. The behaviour of *Leptinotarsa* is also influenced by humidity (p. 304). Tower (1917) took specimens from Chicago and bred them for 9 years at Tucson, Arizona; it appears that in some way the strain became modified by the arid climate to which it was exposed. At any rate, when the insects bred at Tucson were taken back to Chicago, 95–100 per cent. of them failed to survive hibernation, though the mortality in native controls was 10–15 per cent. On the other hand, the Tucson strain, habituated to the arid south-west, could resist the desiccating effects of a stream of dry air much more successfully than the Chicago strain.

Many other examples are on record. For the Japanese rose beetle (*Popillia japonica*, Scarabaeidae), see Payne[1], 1927*b*, 1928, 1929; Ludwig, 1928. For hibernating nymphs of *Chortophaga*[2], see Bodine, 1921, 1923; for *Euxoa segetum* (Noctuidae, Lep.) see Sacharov, 1930. Compare also *Cydia pomonella* (Tortricidae, Lep.), (Townsend, 1926); *Hemerocampa leucostigma* (Lymantriidae, Lep.), (Payne, 1929); *Ephestia kuehniella* (Payne, 1927*a*). According to Robinson (1926*b*), the weevils which infest grain (*Calandra granaria* and *oryzae*; Curculionidae, Col.), are not resistant to cold; coupled with this is the fact that if they are kept at a temperature which makes them dormant, but does not kill them quickly, they do not reduce their water content.

From the examples which I have given, it is clear that ability to survive cold is increased by reduction in the proportion of water in the insect; this may be carried out in the laboratory, and commonly occurs in nature; the examples include a number of insects quite unrelated to one another. It appears probable that the contrary is also true, and that insects which cannot eliminate water when they are put in cold air are not resistant to low temperatures.

If the insect is to survive temperatures much below freezing-point, it must avoid formation of ice in its blood and tissues. The mere reduction in the proportion of water, which is clearly so general, will accomplish much, by concentrating salts and other solutes and so lowering the freezing-point of the body fluids. This is perhaps assisted by the action of an enzyme which works at low temperatures (Payne, 1928; larvae of *Popillia*). But it seems clear that resistance to cold is still further increased, in many insects, by "binding" the water to the colloids in the body, which greatly lowers the temperature at which any portion of the water will turn into ice. The whole problem of bound water is obscure; different techniques applied to the same substance give contradictory results, and I do not think it profitable to discuss it here, particularly as the facts relating to insects have been summarised by Uvarov (1931, pp. 12–14, 69: see also Payne, 1927*b*; Robinson, 1927, 1928*a*, *b*; Sacharov, 1930). The evidence shows that certain insects, if exposed to low temperatures, can bind a large proportion of the water which remains in them after they have eliminated some. Others cannot do so, and are correspondingly much less resistant to cold.

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[1] Payne (1929) refers to larvae losing one-third of their original weight when dehydrated, and not being killed by this loss. In her earlier paper (1928) she refers to a loss of two-thirds of the original weight, but this must surely be an error.

[2] The fact that larvae in this state will gain or lose water according to the atmospheric humidity in which they are kept is referred to on p. 280.

While there is little doubt that the proportion of water in an insect, and particularly the relation between the bound and free water, is of vital importance in relation to hibernation, we have no right to assume that dormancy depends invariably on the quantity or state of the water. It seems clear that the diapause in which many insects pass the winter is not related to water; see, for instance, the data on the corn borer (*Pyralis nubilalis*, Lep.), (Babcock, K. W., 1924, 1927; Babcock and Vance, 1929). The diapause of the larva of *Lucilia* is also not due to humidity, so far as present evidence goes (Roubaud, 1922).

(5) *Behaviour*.

If meal-worms and other insects are kept in a controlled environment, they can maintain a due proportion of water in their bodies by chemical methods (p. 298). But we may suppose that insects regulate their gain and loss of water by reacting to dryness and moisture: in other words, that if an insect is free to choose its environment, its regulation of water content may be by behaviour, as well as by metabolism. As we have already seen, certain insects visit water and drink; others lay their eggs in it, and indeed the great majority of insects lay eggs in moist environments. But though we must suppose that much of the behaviour of insects is determined by perception of atmospheric moisture, we know almost nothing of the anatomical site of the organs which enable the insect to detect water or differences in humidity, nor how they are constructed.

The only relevant work appears to be that by Minnich (1921, 1922). He carried out a series of experiments with adults of two Vanessid butterflies (*Pyrameis atalanta* and *Vanessa antiopa*). In these insects, the proboscis is normally coiled under the head, and it is easy to see when it is partly or entirely uncoiled. The insects can perceive apple juice (but not water) at a distance of an inch or so, and respond by uncoiling the proboscis. The same reaction follows if the under-surface of the tarsus is touched with wet wool. The tarsi can distinguish between water, apple juice, and other substances in solution, and they are not stimulated by dry wool. The response to water is more often obtained in insects which have been in captivity for some days than in those which have not.

But we must assume that many insects possess organs of a different type, which enable them to find water from a distance. We may take mosquitoes, which lay their eggs in water, as an example of insects in which such organs presumably exist. Among the mosquitoes, behaviour in relation to water has been studied in *Aëdes argenteus*, though our knowledge is most imperfect. This insect breeds normally in very small accumulations of water in coconut shells, empty bottles, etc., and the water in these receptacles is highly contaminated with decaying organic matter. The female lays more eggs in water which is contaminated than in pure water. This has been shown in cage experiments by Fielding (1919). Buxton and Hopkins (1927) confirmed and extended this view by working in the field and exposing artificial breeding places which were visited by wild mosquitoes. We showed that contaminated water received more visits from females which wished to lay than did distilled water: moreover, more eggs were laid per visit. The figures given below are typical of our results:

Species	Liquid	Visits	Eggs	Eggs per visit
A. variegatus	Distilled water	23	247	11
,,	Grass infusion	40	639	16
A. argenteus	Distilled water	24	229	10
,,	Grass infusion	25	540	22

It is important to notice that though presence of organic matter increased the attractiveness, a considerable number of eggs were laid in distilled water. The female's reaction to water is further emphasised by Fielding's discovery that if no water and no damp surface is provided, the insect dies without laying eggs.

Many other insects lay their eggs either in water or in very wet material. We do not know whether their behaviour is partly determined by a tropism towards water, or not: but it is certain that it is partly determined by other stimuli. The reader is referred to Adolph (1920) for a discussion of the subject: it is clearly shown that a great range of chemical substances, many of them odorous to man, are attractive to female flies when they are ready to lay their eggs.

From what has been said, it appears that there is very little precise evidence of a tropism towards water: there is more evidence of a tropism towards moist air. But as Necheles (1925) has pointed out, it is very difficult to interpret observations made in nature. According to Savory (1930), in Britain there are two Epeirid spiders of the genus *Zilla*; they are very similar in anatomy, but rather sharply separated in habit and haunt. He took a long cardboard box, put a basin of water at one end and some calcium chloride at the other, and left a spider in it in the dark: no measure of humidity was made. From time to time he opened the lid and noted the position of the spider, removing the web and reversing the lid. *Zilla atrica* was always found over the water at all the temperatures available. *Zilla x-notata* was always above the water at low temperatures, but at 20° C. it was always found over the calcium chloride. Savory suggests that this difference in reaction towards atmospheric moisture may account for the different habitats in which the two spiders are found in nature. Weese (1924) has investigated a similar problem with more complete apparatus. He made use of a machine originally described by Shelford (1913) which gives a gradient of humidity. Air is admitted through a slit in the side of a shallow box, and it is possible to regulate its temperature, humidity and velocity at different parts of the slit. The spider, *Acrosoma rugosa*, always chooses a region of high evaporation, not even avoiding a rate of evaporation greater than any it would meet in nature. Hamilton (1917) made use of a similar apparatus into which he introduced a number of improvements. In it he studied the behaviour of larval and adult Carabidae. The larvae live in soil, and are extremely sensitive to loss of water especially at higher temperatures: they generally seek a region of lower evaporation, avoiding one where it is higher, but many of his larvae got to one end of the experimental trough and remained there. The adult Carabidae behaved in much the same way, but they were less sensitive to evaporation. It appeared also that they were more able to relate their behaviour to the conditions, and to turn away from climate which was unfavourable.

The observations of Breitenbecher (1918) on the behaviour of *Leptinotarsa decemlineata* may perhaps be considered here. In the middle of June, he took a large number of beetles which had been dormant since the previous autumn (p. 300). They reacted positively to light and negatively to gravity. He put some of them in bell jars under different conditions of atmospheric humidity. Those in the moister environment laid eggs; but those which were kept drier did not lay, their tropisms were reversed, and they burrowed in the soil and remained there until rain fell; they then came up and laid eggs. Other beetles of the same lot were buried in soil containing different percentages of water, and the results obtained are shown in Table III. It is quite possible to explain these facts teleologically. One may say that when the beetles are dry, either in the air or in the soil, they react by descending. If they are among growing potato plants, this takes them nearer to the ground and into a zone of lesser evaporation, as Breitenbecher's figures clearly demonstrate. If geotropism takes them into the soil, then again one may presume that a saving of water results. See also observations on harvesting ants (*Aphenogaster barbara*, Hym.), the activities of which are very complex (Buxton, 1924*b*); also on terrestrial Isopods (Allee, 1926), which appear to behave most inconsistently.

Table III. *Effect of burying adult* Leptinotarsa *in soil with different percentages of water.*

Soil water %	15·8	8·8	1·9
Weight, 20 beetles at start (gm.)	3·341	3·329	3·337
Loss weight, % after 8 days	3·8	11·0	33·6
Reaction, start	Light + Gravity –	Light + Gravity –	Light + Gravity –
Reaction, finish	Light + Gravity –	Light + Gravity ±	Light – Gravity +
In breeding cage	Fed, laid eggs	Fed, laid eggs	Buried, till next rain

The experiments which I have quoted throw some light on the insect's power of detecting differences of atmospheric humidity, and directing its movements accordingly. There exists also an interesting body of fact relating to the temperature which insects "prefer"; it seems that the thermotropism of certain insects is regulated in part by the insects' water balance. The experimental method is to provide a gradient of temperature in a long vessel, one end of which is heated and the other cooled. A large number of insects are liberated in this, and their positions noted from time to time. The temperature is read at intervals along the vessel. The vapour pressure of water will be uniform in all parts of the vessel, but the relative humidity and the saturation deficiency will vary inversely with the temperature, but independently of one another. This point has apparently been overlooked by several authors; one writes as if he worked with air which was saturated at all temperatures from 9 to 32° C. (Bodenheimer, 1931 *a*). But setting aside this criticism, it seems clear that the atmosphere in his apparatus was moistened in some experiments and dried in others. The adults of two beetles, *Calathus fuscipes* (Carabidae, Col.) and *Adesmia ulcerosa* (Tenebrionidae, Col.) were entirely unaffected by the alteration in humidity, and assembled round the same optimum temperature irrespective of it. Bodenheimer then kept adult *Adesmia* at controlled humidities for 1–2 weeks, and studied their subsequent reactions when exposed to a temperature gradient. His results were as follows:

Previously at 90 % humidity, preferred 39·4° C. (range 27–47°)
,, 20 % ,, 36·6° C. (range 24–44°)

The numbers used were large, and the experiment was repeated with consistent results (Bodenheimer, 1931*a*). This work is capable of a teleological explanation; one may suggest that the beetles which had been kept in drier air (20 per cent.) needed to economise water, and that by assembling at a lower temperature (and therefore lower saturation deficiency) they were less liable to evaporation[1]. Gunn (1931) has recently dealt with a similar problem, using cockroaches (*Blatta orientalis*, Blattidae), which rapidly lose water and die in a few days. He shows that these insects tend to select a lower and lower temperature as time passes; also individuals which had been previously kept in dry air select a lower temperature than those previously kept in moist air. The proportion of water in cockroaches of a different species (*Periplaneta americana*) at the end of an experiment is lower in those which choose 18–20° C. than in those which choose 25° C. All these observations show that the optimum temperature is affected by the water balance of the insect. If the animal's supply of water, or food, is approaching exhaustion, it seeks a region of lower evaporation (*i.e.* lower temperature). The work of Bodenheimer (1931 *a*) shows that *Zophosis punctata* (Tenebrionidae) behaves in an entirely different manner. In a long series of experiments on adults kept at controlled humidity and subsequently offered a range of temperatures, the following results were obtained:

Previously at 20 %, chooses 35·6–36·95° C. (20–49°)
,, 40 %, ,, 36·3° C. (20–47°)
,, 60 %, ,, 34·8° C. (24–46°)
,, 90 %, ,, 31·9–34·2° C. (19–51°)

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[1] Bodenheimer (1929 *a*) observes that locusts, *Schistocerca gregaria*, choose different temperatures in different months; may this not be a humidity effect?

The experiments are fully recorded and consistent with one another, and the conclusion seems ineluctible. If we are justified in thinking that the behaviour of *Adesmia* and *Blatta* is partly dictated by the need to economise water, then we may say that the behaviour of *Zophosis* is designed to waste it: and that conclusion is improbable, for *Zophosis* runs on the bare baking surface of Palestine in summer.

But whatever "meaning" we may attach to these experiments, there can be no doubt that they raise points of great interest. In future work, hygrometry at different points in the experimental chamber is imperative, and I think it could be carried out with a small weighing hygrometer (Buxton, 1931 *c*); but whatever arrangements are made, any temperature gradient must also be a gradient in saturation deficiency, so that two factors are involved. It appears to me desirable that work should be undertaken, if possible, in a humidity gradient, in a thermostat: the results would be more valuable than any which can be obtained in a temperature gradient, because only one factor would be subject to variation. It is not really legitimate to study thermotropism at all until the effect of atmospheric humidity has been considered, and shown to be negligible.

There are certain observations on humidity which at the moment seem isolated. It is known that the rate at which the snowy cricket (*Oecanthus niveus*, Gryllidae, Orth.) chirps is mainly determined by temperature; but there is some evidence that it is also affected by humidity, the rate of chirping rising if evaporation increases (Allard, 1930; Fulton, 1925; Shull, 1907). In the same category we may put Gill's (1921 *b*, *c*) observation that *Culex fatigans* will not feed on sparrows when the relative humidity is 40 per cent., though they will when it is 50 per cent. or over.

V. THE EGG.

It appears best to treat the egg apart from the other stages. It is not covered with chitin but with a scleroprotein, and it is not tracheate, so that one may assume that the factors which influence its water balance are quite different from those which affect an insect at any other stage. But the distinction is not really as sharp as it appears to be, because an egg which is about to hatch contains a larva which is covered with chitin and tracheate; in certain insects such an egg is capable of resisting conditions which would have been fatal earlier in development.

(1) *Limits of toleration.*

A number of authors have subjected insect eggs to different combinations of temperature and humidity; from their work we may learn something of the relations between the egg and atmospheric moisture.

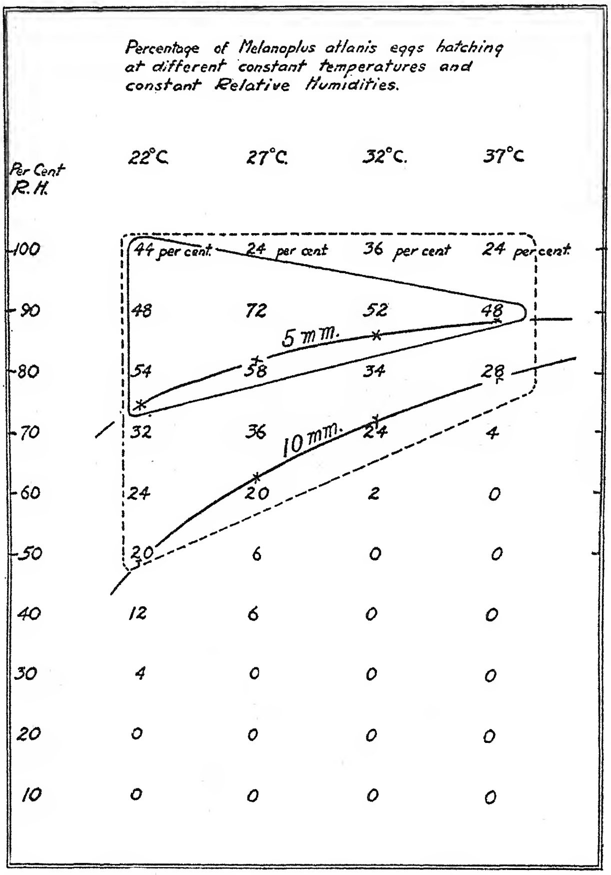
Our knowledge of the eggs of locusts and grasshoppers (Acridiidae) is fairly complete. These eggs are produced in compact packets, which the female buries in soil. Here they may be flooded, or they may be exposed to drought, and the eggs of some species possess considerable powers of resistance to both (see Uvarov, 1931, p. 71). Parker (1930) has made a detailed study of the conditions of temperature and humidity which are tolerated by eggs of the grasshoppers *Melanoplus mexicanus* and

Camnula pellucida. He took a large number of eggs of *Camnula* and put 1000 in each of three pots of soil; in dry soil 69·5 per cent. hatched, in moist soil 91·5 per cent., and in wet soil 72·6 per cent. He took other collections of eggs of both species, stored some of each in dry and others in damp sand, and then exposed them in currents of air of controlled humidity at 23–25° C. Air with a low relative humidity increased the mortality in nearly all his experiments; moreover, eggs of both species which had previously been stored in dry sand were more susceptible to dry air than those previously stored in damp sand. Parker carried out a similar range of experiments at 27 and 37° C., using 100 eggs of *Camnula* in each experiment, and weighing the eggs daily. The loss of weight was very much greater in dry than in damp air; at 10 per cent. relative humidity and 27° C., the batch of eggs lost 56·2 per cent. of their original weight, though ten of them subsequently hatched when placed on dry sand. A similar result was obtained at 37° C., fourteen eggs hatching though the batch had lost 62 per cent. of the original weight. But though some eggs hatched in batches which had lost more than half their weight, one is not justified in assuming that the particular eggs which survived had lost so high a proportion. Even in them the loss was doubtless great, and Parker records that eggs which had actually shrivelled and collapsed, swelled and produced larvae when kept on damp sand. It is clear, therefore, that the embryo of *Camnula* can survive considerable loss of water. This is confirmed by Parker's observation that if eggs of *Camnula* are dried, the thermal death-point rises.

But perhaps Parker's most valuable contribution to our knowledge of insect eggs is one of which he himself was not aware. Working with *Melanoplus atlanis* and exposing eggs to ten different relative humidities at four different temperatures, he obtained the percentage hatches which are shown in Fig. 4: at each combination of temperature and humidity he used 100 eggs, collected in nature. The reader will observe that there is a line surrounding the area in which more than 50 per cent. of the eggs hatched, and another line (shown dotted) surrounding the area in which more than 25 per cent. hatched. These lines, drawn freehand in the original paper, are very close to the lines of equal saturation deficiency which have been added to the figure. We may say, therefore, that the lower limit of atmospheric humidity, consistent with certain percentage hatches, is determined by saturation deficiency, over a wide range of temperature. It will be seen (Fig. 4) that moist air (90–100 per cent.) at 32 and 37° C. is less favourable to these eggs than at lower temperatures. It appears, therefore, that they may be susceptible to water poisoning, as are certain insects (p. 297). Bodenheimer's (1929*a*, *b*) data for eggs of another grasshopper, *Schistocerca gregaria*, appear to be consistent with the same rule. He used seven humidities and eleven temperatures, and carried out experiments at every combination: I have discussed his data elsewhere (Buxton, 1931*b*).

There are two other collections of data which appear to be consistent with the law of saturation deficiency, though neither of them is complete enough to provide a proof that the law can be applied. (See Sweetman and Fernald (1930) on the eggs of *Epilachna corrupta*, and Mehta (1930) on *Dysdercus cingulatus* (Pyrrhocoridae).)

There is a large body of fact on the degree of atmospheric humidity which the eggs of insects will tolerate. Many insects lay their eggs in soil, rotten leaves or similar moist material, but we have few precise observations about the effect of moisture on these eggs. Kerenski (1930) has devoted attention to the egg of *Anisoplia austriaca* (Scarabaeidae, Col.); the egg is laid in soil, and like eggs of several other beetles it swells after it is laid. Eggs known to be less than 24 hours old were exposed to experimental conditions, and weighed individually at intervals. Eggs



Percentage of Melanoplus atlanis eggs hatching at different constant temperatures and constant Relative Humidities.

Per Cent R.H.	22°C.	27°C.	32°C.	37°C.
100	44 per cent	24 per cent	36 per cent	24 per cent
90	48	72	52	48
80	54	58	34	28
70	32	36	24	4
60	24	20	2	0
50	20	6	0	0
40	12	6	0	0
30	4	0	0	0
20	0	0	0	0
10	0	0	0	0

Fig. 4. Number of hatches from 100 eggs of *Melanoplus*, exposed at each point in the diagram. The table is Parker's: across it have been drawn lines of equal saturation deficiency. (Buxton, 1931 *b*, with acknowledgments to the Entomological Society of London.)

laid in damp soil doubled their weight during the first week. The same increase took place on filter paper moistened with an aqueous extract of soil, or with distilled water. The gain in weight is not simply due to osmosis, for it takes place in solutions of sodium chloride up to 4 per cent., also in 2 per cent. potassium nitrate, and 2 per cent. barium chloride. The egg is presumably covered by a membrane which excludes the salts, but actively absorbs water from them.

Kerenski also discusses the swelling of eggs of Sawflies (Tenthredinoidea, Hymenoptera); these eggs are inserted by the female in a slit which she makes in plant tissues, and they swell after they are deposited: it is possible that the newly laid egg is feeding by osmosis, or that during metabolism the material which was in the egg at the time of deposition comes to occupy more space than it originally did. Peacock's (1928) experiments carry the matter a little further. He took twigs of gooseberry, within the leaves of which *Pristiphora pallipes* had laid eggs, and placed the cut ends of the twigs in eosin solution: the dye passed into the egg. But when he dissected eggs of the same species from the body of the female and stuck them on the surface of the leaves of the plant, a proportion developed into larvae. It appears therefore that the egg of this insect normally absorbs liquid from the plant tissues, but that it is not necessary that it should do so.

The weevil *Sitona lineata* lays eggs on the leaves of peas. Their limits of toleration have been studied by Andersen (1930). Exposing eggs to many humidities and temperatures between 8 and 20° C., he showed that the highest proportion of hatches took place between 72 and 82 per cent. relative humidity. Below 70 per cent., the proportion of hatches fell and at 62 per cent. only three eggs out of thirty-nine produced larvae. In saturated air, there was also a higher mortality than at a humidity of 82 per cent., if we may trust a single experiment on a small number of eggs. Zwölfer's (1931) observations on the egg of *Panolis flammea* (Noctuidae, Lep.) show a wide range of toleration, but total mortality in saturated air. The observations of Holdaway (1928) on the egg of *Tribolium* are similar. On the other hand the eggs of many insects require a very high degree of humidity, and are not harmed by saturation: for instance, *Prodenia* (Noctuidae) (Janisch, 1930); *Haematopinus* (Anoplura) (Bacot and Linzell, 1919); *Tomaspis* (Cercopidae, Rhynch.) (Urich and Pickles, 1930); *Sminthurus* (Collembola) (Davies, 1930); *Mayetiola* (Cecidomyidae, Dipt.) (Headlee and Parker, 1913); *Xenopsylla* (Plague Commission, 1912). The egg of *Aphis avenae* and *A. pomi* is easily killed by drying, but the position is complex as there is an outer shell, resistant to drying but liable to split (Peterson, 1920). The egg of *Lachnosterna* (= *Phyllophaga*, Scarabaeidae, Col.) is unfavourably affected by dry soil (Sweetman, 1931*a*): the effect may be on the egg, or related to the mechanical differences between dry and wet soil.

Discussion. In almost every example a larger number of eggs hatch in air which is at or near saturation, than in air which is drier. I have not found any record of eggs which produce a higher percentage of hatches if they are kept in dry air; eggs of *Panolis* and *Tribolium* and perhaps that of *Sitona* are unfavourably affected by saturated air. One may well wonder what is the precise cause of this. Is dry air fatal because it causes a loss of water from the embryo and kills it, or because it causes the eggshell to become hard and impenetrable? Few authors have devoted attention to this point. Bacot and Linzell (1919) state that when the egg of *Haematopinus asini* is exposed to dry air, the embryo dies before development has proceeded far. Presumably the cause of death is the same, whenever eggs, which have been exposed to dry air, shrivel and die. But in other cases, there is no doubt that death is due to the inability of the larva to penetrate the shell. Janisch (1930) states that this is so when eggs of *Prodenia littoralis* are exposed to air with an unfavourably low humidity. Severin and Severin (1910) clearly demonstrated the same cause of death in the stick insect, *Diapheromera femorata* (Phasmidae).

Inasmuch as the optimum for so many eggs is at or near saturation, it is certain that the water formed during metabolism accumulates inside the egg shell: whether it does so in the growing tissues, or elsewhere, is not known.

(2) *Dryness causing dormancy.*

The eggs of some insects are tolerant of drying, though they remain dormant in dry air and only hatch when they are wetted. Payne (1929) states that eggs of the moth *Hemerocampa leucostigma* can be kept for as much as 2 years over calcium chloride, and that if they are soaked with water they will then hatch. Goodwin (1922) has recorded the survival of eggs of *Tenebrioides mauritanicus* (Trogositidae, Col.) for 20 months among dry corn in a stoppered bottle. Lounsbury (1915) states that the eggs of the South African locust, *Locusta pardalina* (Acridiidae), which are normally laid in moist soil, hatch in 14 days in summer: but if they are dried, development is suspended, and he has known eggs to hatch which have been kept for 3½ years. Prof. J. C. Faure informs me by letter that he has hatched eggs of the same species which had been kept dry in his laboratory for 3 years and 1 month; only 2–4 per cent. of the eggs hatched: if eggs which have been dried are wetted and incubated, no hatch occurs for about 10 days: the stage which is resistant to drying is one in which the egg contains a very small embryo.

The eggs of the springtail, *Sminthurus viridis* (Collembola), possess remarkable powers of resisting drought without dying; but they only hatch in saturated air (Davies, 1930; Maclagan, 1932). According to Holdaway (1927), the egg is fluid at the moment it is laid, and it becomes firm and spherical when exposed to the air. If eggs are kept moist, the young springtails emerge in about 8 or 10 days, but if the soil becomes dry, the eggs collapse completely. In this condition they can be kept for at least 271 days without dying. If dried, collapsed eggs are wetted, they become spherical very quickly, but they only produce young springtails after a period of 12 days or more. The recent work of Davidson (1932) has shown that the egg is most resistant to drying when it is about half incubated. The freshly laid egg dies in dry soil, and so does the egg which is nearly ready to hatch: in this case death is perhaps due to the egg swelling and the chorion splitting. These facts are particularly interesting, for the insect itself is easily killed by drought (Davies, 1928), and it is quite clear that the species exists in a large part of its range because the eggs are capable of survival in dry weather.

The eggs of the yellow-fever mosquito, *Aëdes argenteus*, are able to resist drying in certain circumstances. The female deposits her eggs at the edge of water in a tree-hole or an old bottle. Providing that they remain wet, the eggs produce larvae in about 48 hours in a tropical climate. But even under natural conditions, if the eggs remain wet a proportion only hatch after a long delay. MacGregor (1916) has shown that, if drying occurs during the course of the development of the embryo, it is fatal: when development is complete, drying causes no mortality. Mature eggs do not shrink when dried, and eggs which shrink are dead. Mature eggs are capable of resisting death for very long periods. Bacot (1918), working on eggs kept between 7 and 18° C. in moist air, showed that after 7 months' storage the emergence was still over 80 per cent. After that period, it fell steadily; after 13½ months, he only obtained one larvae from about 1000 eggs, and in several experiments which lasted longer, he obtained no emergencies at all. The data published by Fielding (1919) are not dissimilar. Working in Samoa (Buxton and Hopkins, 1927), we were able to show that the normal hatch of eggs which had not been dried was over 90 per cent. We took mature eggs of this species and exposed them over strong sulphuric acid in desiccators at a temperature of 27–28° C. At the end of a certain number of days, during which the controls had been kept dry in the laboratory, they and the experimental eggs were put into dirty water[1]. Our results may be summarised as follows:

	Hatch %					
	2 days	3 days	14 days	21 days	30 days	40 days
Relative humidity 0%	100	53	43	62	4	3
Control	98	100	100	93	57	59

Many other mosquitoes of the genus *Aëdes* and of other genera lay eggs which are resistant to some degree of drying, but I think that experimental work has only been carried out on *Aëdes variegatus* (Buxton and Hopkins, 1927).

Very much more work requires to be done on the eggs of *Aëdes*. We do not know whether their eventual death when they are dried is due to exhaustion of water or of food. But it appears probable that they die of exhaustion of food, because those which are stored on blotting paper under ordinary conditions of temperature and humidity survive about as long as those which are stored in distilled water—a fluid in which only a small proportion will emerge. But even if it is the exhaustion of its food supply which eventually kills the egg of the yellow-fever mosquito, it is clear enough that it has remarkable powers of resisting desiccation. Though the newly laid egg is not water-tight, one must assume that it becomes so during development[2]; first, because a normal larva, not apparently shrivelled, can be found within it if a dried egg is dissected; secondly, because larvae frequently emerge in a few minutes when dry eggs are put in dirty water.

The phenomenon of resistance to drying in the egg of *Aëdes* is clearly unlike that presented by *Sminthurus*, for in that insect the eggs collapse when they are dried though they do not die; and they only hatch after a prolonged period when they are wetted. It seems certain that in *Sminthurus* the actual tissues are tolerant of loss of water. The egg of *Camnula* (and probably *Locusta*) is evidently similar to that of

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[1] At the time it was done, this control appeared to be satisfactory. But it is always possible that the sulphuric acid, which has a definite though low vapour pressure, will act unfavourably on insects kept over it, though it is known that many insects are not so affected. If the work is repeated, the control eggs should also be in a desiccator, over a mixture of acid and water which gives a moderate humidity, say 70 per cent.; or else the experimenter should use a different drying agent.

[2] Compare the fact that unfertile Lepidopterous eggs generally shrivel within a few days; fertile eggs kept with them do not.

Sminthurus; it can recover by hygroscopy after it has collapsed; eggs which have been partly dried have a higher thermal death-point than those which have not. With regard to the other insects, the eggs of which become dormant when they are dried, for instance *Hemerocampa* (above), we have no information. We do not know whether the shell is water-tight, or whether the tissues are capable of remaining alive when they are dried.

(3) *Humidity and duration of the egg stage.*

We have discussed eggs which are not killed by drying, though their emergence (and in some cases their development) is delayed until they are moistened. There are other eggs, which continue to develop over a wide range of humidity, though it affects the rate of development[1]. Janisch (1930) showed that the development of eggs of *Prodenia littoralis* at 29° C. occupied 60 hours at relative humidities between 0 and 26 per cent. With rising relative humidities the period of development became regularly less and less, being 49 hours at 75 per cent., under 48 hours at 96 per cent., and 43 hours in saturated air. He performed similar experiments at higher temperatures up to 40° C., and showed that a low relative humidity always delayed the emergence of the larva from the egg: but at the higher temperatures the shortest periods were recorded in air of which the relative humidity was 86–96 per cent.; the duration was definitely longer in saturated air. Wardle's (1930) data on the duration of the egg stage of the greenbottle fly (*Lucilia sericata*, Muscidae) gave a similar result. Andersen (1930) has made a full study of the effect of humidity upon the eggs of the weevil, *Sitona lineata*. Working between 16 and 20° C., he carried out five separate experiments, exposing eggs to relative humidities from 62 to 100 per cent. His data for the duration of the egg stage, in days, may be summarised in this way:

Temp. ° C.	Relative humidity (approx.)									
	100	82	77	75	72·5	71·5	69·5	66	64	62
16·0	17·6	19·0	20·6	20·5	20·3	20·8	22·0	22·4	—	—
18·3	13·7	14·0	15·0	15·0	16·6	17·6	18·5	19·6	20·0	22·0
19·9	10·7	—	—	—	13·4	16·2	16·0	15·5	19·2	21·0

It will be seen that there is a consistent lengthening of the duration of the egg stage at the lower relative humidities. The same phenomenon is shown clearly for the egg of *Lasioderma* (Anobiidae, Col.) (Powell, 1931) and *Epilachna* (Pyenson and Sweetman, 1931). For less complete records of a similar nature, see Headlee and Parker (1913), Hunter and Pierce (1912).

In every example which I have quoted, the effect of dry air is to lengthen the duration of the egg stage: but this is not universally true. Holdaway (1928) showed that the duration of the egg stage of *Tribolium* is entirely unaffected by humidity, at 27° C. Headlee (1921) says that the length of the egg stage of the bean beetle (*Bruchus obtectus*) is 6 days in saturated air and 4 days in air with a relative humidity of 24 per cent. But before we can assess the importance of this, we must have a more complete collection of facts collected at several humidities and several temperatures: it is possible that the egg of this insect is scarcely tolerant of saturated air, and that under these conditions, which are so artificial for it, development is prolonged. Sweetman's (1931*a*) data on the duration of the egg stage of *Phyllophaga*, when stored in soil holding different proportions of water, seem anomalous; we do not know the number of eggs employed, or the consistency of the results. Hennings (1907*a*, *b*) showed that the egg stage of *Ips typographus* (Ipidae, Col.) is shorter in drier air, at three out of the four temperatures tested: his methods and results have been criticised above (p. 292). Parker's (1930) observations on the eggs of the grasshoppers *Melanoplus mexicanus* and *Camnula pellucida* are difficult to interpret. At 23–25° C. and also at 37° C., he showed that low humidity delays emergence. But at 27° C. he obtained contradictory results with *Camnula*. The explanation perhaps lies in the fact that he was working with eggs of unknown age, collected in the field.

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[1] It should perhaps be stated that erratic emergence, which is characteristic of the eggs of many insects, is not apparently connected with the humidity of the air in which they are kept. Indeed it is a matter of common observation that a number of eggs, all laid by one female at a time, may emerge over a period many times longer than that occupied by the shortest of them. A few examples of such erratic behaviour selected from the many which are on record, can be found in Urich and Pickles (1930) (second brood of *Tomaspis* erratic, first not); Parker (1930) (p. 10, *Melanoplus* eggs erratic at many temperatures); Bacot and Linzell (1919), Buxton (1930*b*).

Discussion. There is conclusive evidence that the length of the egg stage of several insects is greater in dry than in moister air of the same temperature: this is clearly shown by the eggs of *Prodenia*, *Lucilia* and *Sitona*, which are members of three different orders. Moreover, the phenomenon has been shown to occur at several different temperatures for each insect. *Tribolium* is an exception and there are apparent anomalies in the eggs of *Bruchus*, *Phyllophaga*, *Ips*, *Melanoplus* and *Camnula*: but these may be due to technical difficulties and will perhaps disappear when knowledge is more complete. It may be held that the majority of the facts lend support to Bêlehrádek's hypothesis (1926, 1930). According to his view, the speed of development and indeed of many other natural processes is determined by the viscosity of protoplasm. The viscosity of protoplasm is in turn largely determined by the proportion of water in it. We should be more able to accept or refute this view if we could show that exposure to dry air reduces the proportion of water inside the eggs. But we have at present no estimation of the water content of any of these eggs either before or after exposure to controlled conditions, though there is presumptive evidence that the embryo of *Camnula* can lose a large proportion of its water without dying.

VI. SUMMARY.

The gain and loss of water by insects is discussed, also the total amount of water in the insect's body. The paper does not deal with the water content or the osmotic balance of particular organs or tissues: neither does it discuss the movement of water within the insect's body. The subject is on the borderline, where physiology appears to extend and interpret ecological observations.

The majority of insects do not drink, but rely largely on the water which is contained in their food. Insects which breed in dry material or live in deserts must be

able to resist loss of water, and water formed in metabolism is of great importance to them. In the fasting meal-worm, metabolism is so adjusted as to produce as much water as is lost by evaporation: this in turn is proportional to the saturation deficiency, at any rate at 23° C. Several insects can gain water from an atmosphere which is nearly saturated. It is difficult to explain this on physical grounds: the vapour pressure of the tissue fluids, including the liquid in the tracheoles, is so close to the saturation vapour pressure of water that condensation into the insect could only occur if the external atmosphere was within 1 per cent. of saturation. Perhaps there is a secretion of water into the body of the insect: this explanation is difficult to accept at first sight, but such secretion would be no more remarkable than the activities of many types of gland.

Loss of water is partly by diffusion from the respiratory system. It also takes place from the surface of the body in some insects, but apparently not in all. It is known that the duration of life, or the loss of weight during starvation, of several insects is proportional to the saturation deficiency. This is only true within certain limits: these are reached when the saturation deficiency is either very great or very small. Many insects can reduce their temperature below that of the surrounding air, at least when they are put in air which is fairly dry and above 20° C.: this is presumably due to evaporation. The thermal death-point is also affected by evaporation. It may be lower in dry air, presumably, owing to excessive loss of water: or it may be higher in dry air, showing that the insect can cool its body by evaporating water—at any rate for a short period. Some insects do not lose water at all, and there is reason to believe that efficient cooling by evaporation is only possible for a relatively large insect: a small insect, in which the ratio of surface to volume is great, gains so much heat by convection, that if it were to compensate by evaporation it would die of desiccation in a very short period.

Certain insects can maintain a particular proportion of water in the body even if external conditions change widely, but other insects lose a large proportion of their water without being killed. The normal water content alters with growth, metamorphosis, and other factors. In insects which normally hibernate, a large proportion of water is lost before dormancy. This in itself presumably lowers the temperature at which the tissues would freeze: danger of death from freezing is also reduced in many insects by binding a large proportion of water to the colloids of the body. The maintenance of a due proportion of water in the insect is partly carried out by chemical methods, but it is also due in part to behaviour. Certain insects transfer themselves to regions of less evaporation when the air is dry, or when a material proportion of water has been evaporated from their bodies.

The existence of an insect in a very damp atmosphere, which is the normal environment of many of them, depends on the excretion of water through the Malpighian tubes, and on the passage of damp faeces. But a large number of insects, even among those which require a moist environment, are killed by exposure to saturated air. It is supposed that insects can only exist under very dry conditions if they possess several qualities in combination. Loss of water from the alimentary canal must be almost nil: this is assisted by the excretion of solid uric acid, and by efficient extraction of water from the contents of the hind-gut. Certain insects also appear to reduce the loss of water through the skin to a very low figure. It is assumed that they have no control of the diffusion of water from within their tracheal system.

The relation of a particular insect to atmospheric moisture is often very precise; moisture must often be a determining factor. The conditions which are most favourable may perhaps be defined in this way. If low humidity is unfavourable, then the higher the humidity the better, up to the point where elimination becomes impossible: in fact, the optimum is just below the point of danger. Similarly, if growth and reproduction are to be as rapid as possible, the temperature must be just under that which is harmful.

The insect's egg may be regarded as a separate problem. Certain eggs can tolerate a considerable loss of water from their contents. In some eggs, at any rate, loss of water is directly proportional to saturation deficiency. The eggs of many insects occur normally in places where the humidity is very high: most of them do not suffer from exposure to saturated air, though a few are known to do so. The death of the egg in air which is too dry is sometimes caused by the death of the embryo, at others by the shell becoming so hard that hatching is impossible. Certain eggs tolerate dryness, which causes them to become dormant. They fall into two classes. In one class, toleration to drying may occur early or late in development, and the embryo itself loses water. In the other class, dryness is only tolerated when the egg is ready to hatch: the larva within it does not lose water, but the shell of the egg becomes water-tight. In many insects, exposure to a low but not fatal degree of humidity increases the duration of the egg stage.

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The list given below includes all papers to which reference is made in the text; a few papers of potential value which do not appear to be related to the subjects discussed have been gathered together as "supplementary references." I have not included a number of observational and ecological papers, which can be found in the very extensive bibliography appended to Uvarov's paper.

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UNIT CHARACTERS IN FOSSILS

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(With Four Text-figures.)

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I. INTRODUCTION.

THE term "unit character" is one which has played a prominent part in the literature of experimental biology. Though it has fallen into disrepute (Morgan *et al.*, 1915), it still serves the useful purpose of supplying a name for the simplest of those physical features which make up the body of an organism. H. F. Osborn (1917) has suggested "biocharacter" as an alternative term for these visible units, the study of which opens the way to an understanding of the hereditary processes. Hitherto the palaeontologist, like the comparative anatomist, has been mainly concerned with the more complex units represented by the organism as a whole, and by its organs and structures. Nevertheless he has, incidentally, recorded a multitude of observations upon the simplest units, the unit characters. These throw a flood of light upon certain problems which also interest the geneticist, but which are being approached by him from a different starting-point. The latter worker studies evolutionary changes at their inception, the former follows them as they proceed in full swing across the ages. Sooner or later these two must find themselves on common ground where their conclusions may be correlated and the palaeontological counterparts of genetic phenomena may be discovered.

Though the palaeontologist cannot, like the geneticist, follow his unit along carefully controlled lines of descent, he can trace it across successive intervals of time, from its manifestations as they are seen in the members of a species living at one period, to its condition as seen in closely allied forms living at slightly later periods. In discussing the problems arising out of his observations in the following pages the term "community" will be used in the sense of a pure community, that is to say one which consists of individuals of the same or very closely allied species. This is in contradistinction to the common practice of using it for a mixed community, that is to say one whose members belong to a number of quite unrelated species. Since, under natural conditions, the members of a pure community breed uncontrolled with one another, the geneticist's method of enquiry must be supplemented by that of the palaeontologist if fuller light is to be gained upon the broader problems of evolution.

The "imperfection of the palaeontological record," which is due to breaks in the sequence of the rocks and to the existence of considerable thicknesses of unfossiliferous strata, seemed, for a long time, to be an insuperable barrier. In some instances, however, these gaps have been successfully bridged, and the fact that the communities they separate belong to the same evolving stock has been established. In descriptive accounts of such work two terms—lineage and gens (Vaughan, 1905)—are frequently used, and their significance must now be briefly indicated. Since the members of a community inter-breed freely a succession of communities is made up of a plexus (Trueman, 1924; Bather, 1927), of intertwining lines of descent. The term "gens" covers the whole plexus of descent, and includes all the variations exhibited by the members of each community in the series. On the other hand, "lineage" has been commonly used for a selected series of normal individuals exhibiting the main sequences of developmental change manifested by successive communities. Recently there has been a tendency to broaden its significance and to regard it as more nearly synonymous with "gens" as defined above. In the following pages it will be used in its earlier significance.

II. THE EVIDENCE FOR THE VALIDITY OF A GENS.

The requisite evidence for linking together communities separated by intervals of time is furnished both by the individual and by the whole community.

In dealing with the evidence furnished by the individual the procedure is that universally adopted by systematists, which is based upon the principle that similarity of structure implies relationship, and that degree of similarity is a measure of the closeness of relationship. It is assumed that these principles are as true for individuals distributed in time as in space.

To those who are accustomed to dissecting and investigating the soft, as well as the hard parts of animals, the shells of such organisms as Mollusca and Molluscoidea appear to possess too few characters to provide a reliable foundation for establishing relationships. But those who have only the hard parts at their disposal find that the simplicity is merely apparent. Reference to the literature discussed in the following pages will show that intensive study of detail reveals a sufficient number of points for comparison to establish quite close biological relationship.

Perhaps the most unpromising group is the Pelecypoda. Nevertheless even among them the quest for satisfactory evidence is far from being hopeless, as may be seen from a consideration of the genus *Gryphaea*. A. E. Trueman (1922) when investigating the evolution of *Gryphaea incurva*[1] measured numerous individuals collected from four horizons. He mentions six characters as important, though he does not rely exclusively upon these. The first is the arching of the left valve. A brief consideration of all that is involved in the production of such a curve will suffice to satisfy the most exacting that the chances are very remote that this curve would be repeated independently in quite unrelated stocks. The same is true also for the other characters he mentions. When these six characters together with certain sequences in their behaviour are to be repeated in combination the likelihood of independent repetition is still more remote.

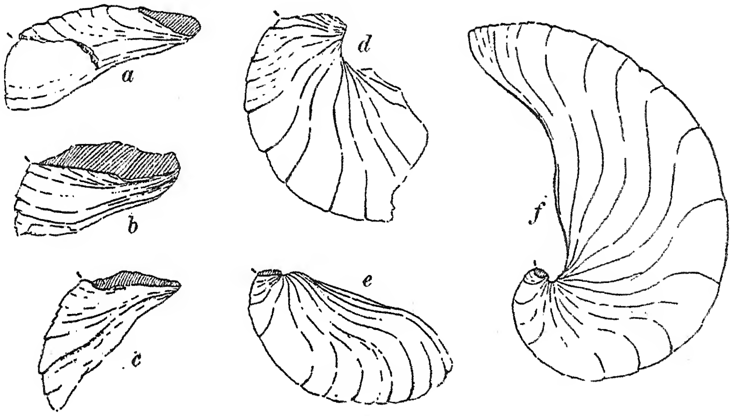

Fig. 1. Left valves of representative examples of the gens *Ostraea irregularis—Gryphaea incurva*. *a–c*, varieties of *Ostraea* cf. *irregularis*: *a*, this approaches *O. liassica* from a lower horizon; *c*, an advanced form. *d*, *Gryphaea* cf. *dumortieri* approaching to *G. obliquata*. *e*, *G.* aff. *obliquata*. *f*, *G. incurva*. Area of attachment shown by shading. The short stroke indicates the approximate position of the passage from ostraeoid to gryphaeoid phases in development. Note the close similarity of the umbonal portions of *d–f* to the adults *b*, *c*.

Trueman describes these six characters as "progressive." Thus, for example, the arching shown by the later shells is that of the earlier plus a little more. The close similarity of the first section of the arch in later shells (Fig. 1), to that of the whole of an earlier shell, is a portion of the evidence upon which the relationship of the later to the earlier is established. The same type of phenomenon is exhibited by the other characters. Any taxonomist handling an adult specimen of *Ostraea irregularis*, and very young specimens of *Gryphaea dumortieri*, *G. obliquata* and *G. incurva*, would be compelled, by the closeness of their similarity to one another, to conclude that apart from size they were specifically identical.

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[1] My thanks are due to Dr Trueman for placing representative examples of this gens at my disposal and for helpful criticisms and suggestions relating to this article.

The individual *G. incurva* during its growth passes from specific identity with *Ostraea irregularis* of an earlier period to a combination of character phases so different that the use of another specific, or even generic, name is justified. The lineage *Ostraea irregularis—Gryphaea incurva* is not a phylogenetic erection built upon any theory of recapitulation, it is a series of individuals whose very close biological relationship has been established upon accepted taxonomic principles, in which the possession by these organisms of a cluster of characters exhibiting many of the same phases in development is recognised.

The evidence based thus upon the detailed structural comparison of individuals is supplemented by a comparison of successive communities of the same or similar species. Trueman shows that the arching of the shell in different individuals of the gens to which *G. incurva* belongs varies from only a few degrees up to as much as 540°. The range of variation exhibited in any one community, however, coincides with only a portion of the range for the whole gens. Had there been no overlap of the ranges of variation in successive communities the evidence for relationship between them would have been limited to the fact that the ranges when placed end to end formed a continuous series. But the ranges were as follows:

	Range in degrees	Amount of overlap
Level No. 5	270–540	230
,, No. 4	220–500	180
,, No. 3	180–400	160
,, No. 2	100–340	100
,, No. 1	10–130	—

The existence of this overlap in the ranges of variation of successive communities can be accounted for only by assuming that so far as this character is concerned they possess a common heritage. A similar study of the other characters would be of great value in establishing the unity of the heritage possessed by these communities.

Though the above discussion is based upon specimens collected from only four horizons, it is highly probable that intervening levels will yield transitional material. The facts as they stand, however, furnish an example of the type of evidence which can be used to bridge across minor gaps in the stratigraphical record. Despite the existence of such gaps, the sequence of changes which is the subject of this enquiry is fully known, for it is recorded in the deposits laid down by the organism itself in its own shell. The evidence also shows that such fossil remains were produced by very closely related animals, that is to say by animals having a common heritage.

As already noticed the range of variation, exhibited by successive communities, shifts consistently from arching of low to one of high degree. The mode likewise shifts in the same direction. These facts imply a heritage that is changing its degree of expression, that is to say it is evolving.

Though the later communities have evidently been derived from the earlier it cannot be said of any one specimen that it is the direct descendant of another from a lower level. It is therefore advisable to avoid the use of the term "genetic" in connection with the relationship of fossils to one another, and to substitute the term "biological" in its stead.

Such a gens or evolving community series as that discussed above—established by the study of many specimens collected from successive known geological horizons, and exhibiting a sufficiently close similarity of growth stages and of variations to bridge across gaps caused by stratigraphical breaks and barren strata—furnishes suitable material for the study of unit characters with a view to gaining light upon their behaviour in evolution.

III. EXAMPLES OF OTHER GENTES.

The number of cases in which the working out of the history of a gens fulfils the conditions laid down above is unfortunately very small. The work of R. G. Carruthers (1910) upon "The evolution of *Zaphrentis delanouei*" does so nearly perfectly. In this gens he takes certain clearly recognisable phases in the change of form exhibited by the fossula as landmarks in the evolutionary series. The fossula is, however, a complex feature which owes its changes of form in development and evolution to the independent behaviour of several distinct unit characters, viz. four pairs of septa, and a quantity of lime deposited at their inner ends. One of the two septa immediately adjoining the cardinal septum may be taken as the character to be studied. In *Z. delanouei* (*s.str.*) its length increases during life up to half that of the radius of the coral. In *Z. parallela* it becomes longer. In *Z. constricta* it is almost as long as the alar septa and extends nearly to the centre of the coral. In successively later communities of *Z. disjuncta* it shortens until it is almost on the verge of disappearance.

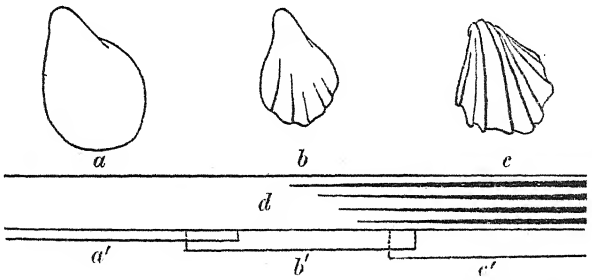

Fig. 2. Diagram representing the progressive phases in the development and evolution of sulcation in the gens *Inoceramus concentricus—sulcatus* (based on Woods). *a*, *Inoceramus concentricus* (Lower and Upper Gault). *b*, *I. concentricus* var. *subsulcatus* (lowest subzone of Upper Gault). *c*, *I. sulcatus* (Upper Gault). *d* represents the onset and gradual expression of sulcation in evolution of the gens during the Gault period. The lines *a′*, *b′*, *c′* indicate that portion of the evolutionary history of sulcation to which the phases shown in the development of *a*, *b*, *c* run parallel.

Work upon other gentes often lacks the statistical detail supplied by Trueman and Carruthers. This defect is partially compensated for by an ample supply of plates and text-figures and by general conclusions based upon the examination of a great many specimens. An example of this type is furnished by the work of H. Woods (1910, 1912) upon *Inoceramus*. In the Lower Gault of Folkestone the species *I. concentricus*, characterised by a smooth shell, occurs. In the Upper Gault it is accompanied by *I. sulcatus* in which the shell is ornamented with folds radiating from the umbo to the margin. In the lowest zone of the Upper Gault *I. concentricus* var. *subsulcatus* is also found. In this the shell in youth is as smooth as it is in the adult of *I. concentricus*, but in middle and later life it becomes partially or wholly plicated as in *I. sulcatus*. Taking one fold as a unit character it is seen to appear in this variety for the first time, almost imperceptibly, in middle life and develops fully in later life.

In discussing the gens *Z. delanouei* it was seen that the septum in the late phases of its history underwent retrogression. Though this phenomenon is a very common one, it is unfortunately difficult to find an example which culminates in the complete disappearance of the character and at the same time fulfils the conditions imposed above. In the absence of a better example therefore, the work of Amadeus W. Grabau (1904) upon *Clavilithes* will be taken as illustrative. As pointed out by A. G. Wrigley (1927), in a discussion on the biological series—*C. rugosus*, *C. dameriacensis*, *C. conjunctus*, *C. parisiensis*, *C. subscalaris* and *C. scalaris*—the stratigraphical evidence, as it stands at present, is far from satisfactory. Nevertheless he agrees "with the theory that *Clavilithes* had a 'fusoid' ancestry." The degrees of departure from that primitive type are exemplified by the series of species mentioned above. The character which may be taken for study is the transverse rib. This is present at all stages of growth in *C. rugosus*. Young specimens of *C. dameriacensis* are identical in this respect with *C. rugosus*, but in late life the ribs fade away. In *C. conjunctus* and *C. parisiensis* this character is present in early life, but absent in

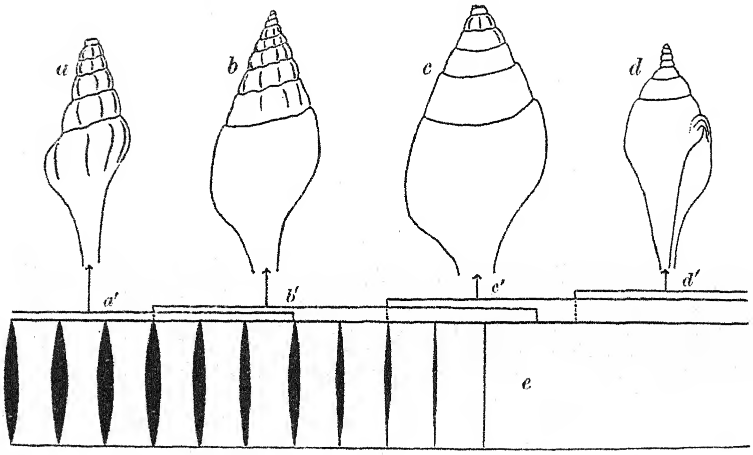

Fig. 3. Diagram representing the retrogressive phases in the development and evolution of transverse ribbing in the biologically related series *Clavilithes rugosus—scalaris*, from the Middle and Upper Miocene of the Paris Basin (based on Grabau). *a*, *C. rugosus*. *b*, *C. dameriacensis*. *c*, *C. conjunctus*. *d*, *C. subscalaris*. *e* represents the gradual decline in expression and ultimate disappearance of transverse ribbing during the evolution of species of the genus *Clavilithes*. The lines *a′*, *b′*, *c′*, *d′* indicate that portion of the evolutionary history of transverse ribbing to which the phases shown in the development of *a–d* run parallel.

middle and late life. In some varieties of *C. subscalaris* and *C. scalaris* this feature has dropped out of development completely.

There are numerous other examples of similar work upon lineages and gentes in other sections of the Invertebrata, but these are the only ones to which reference will be made in the following pages.

IV. THE CHARACTERISTICS OF THE PALAEONTOLOGICAL UNIT.

As a preliminary to attempting to link up the palaeontological study of unit characters with that of the Mendelian experimenter a summary of the peculiarities of the unit as revealed by the study of its behaviour in well-established gentes may be given.

(1) The unit character undergoes serial change (Osborn, 1907; Bather, 1920), both in development and evolution.

All the unit characters referred to above undergo serial change. In some cases (*e.g.* the sulcus in *Inoceramus*) this takes place progressively from an almost imperceptible inception[1] to a full expression. In other cases (*e.g.* ribs in *Clavilithes*) it is retrogressive from a full expression to ultimate disappearance.

(2) Serial change in development is parallel to that in evolution.

One advantage that fossils present in the study of unit characters is that the phenomena exhibited during development may be compared with the series of changes exhibited in adult individuals of successive communities. Such comparison reveals a striking parallelism between the two. This may be regarded as recapitulatory or anticipatory according as the view-point adopted is at the end or the beginning of the series. Thus, for example, in late members of the *Gryphaea incurva* gens the degree of arching attained in youth and adolescence is the same as that exhibited by the adults of *Ostraea irregularis* and *Gryphaea dumortieri*, whilst that attained in maturity in *O. irregularis* anticipates the condition which becomes dominant throughout life in the various species of *Gryphaea*.

(3) The time of onset of a character and of its successive phases of change varies (Bather, 1920) in different individuals and changes progressively in successive communities (cp. Table I).

Thus the gryphaeoid type of arching of the shell begins in late or middle life in different varieties of *Ostraea irregularis*, in adolescence in *Gryphaea dumortieri* (No. 3), and in very early life in *G. incurva*.

Further, the size at which individuals attain 50° of arching ranges from 26 mm. in *Ostraea irregularis* to 2½ mm. in *Gryphaea incurva*.

(4) The rate of change in expression of a character varies in different individuals, but becomes progressively more rapid in successively later communities.

Thus in representative examples from the *Gryphaea incurva* gens when the length of the last-formed layer of shell is 26 mm. the degree of arching is that shown in column A in Table I.

(5) Unit characters behave independently of one another (Spencer, 1914; Lang, 1920).

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[1] Osborn (1907) introduces the term "rectigradation" for this.

Table I.

	A	B	C	D	E
1	20	1·3	26·0	0·8	0·6
2	75	2·9	18·0	1·1	4·7
3	150	6·2	12·5	3·2	8·2
4	210	8·3	2·9	3·7	8·6
5	250	9·2	—	—	—

1–5. Specimens used in making the above measurements.
1. *Ostraea irregularis* (with features approaching *O. liassica*).
2. *O. irregularis* (typical).
3. *Gryphaea dumortieri* (with features approaching to *G. obliquata*).
4. *G. incurva* (typical).
5. *G. incurva* (more advanced).

Nos. 1–4 are shown in Fig. 1 *a*, *c*, *d* and *f* respectively.

Columns A–E. Series of measurements made upon specimens 1–5.

A. Degree of arching attained by each specimen when the length of the lamellae of which the shell is built was 26 mm.

B. The average increase in degree of arching per millimetre increase in length of lamellae for each specimen.

C. Length of lamellae at the time of onset of gryphaeoid arching.

D and E. Average number of degrees of arching per millimetre of length of lamellae before (D) and after (E), the onset of the gryphaeoid type of arching.

This is indicated by the fact that phases in the expression of a character, time of onset of expression, and of its phases, rate of change of expression during development and evolution, are not determined by those of other characters. This may be illustrated by a consideration of the arching in the *Gryphaea incurva* gens in relation to growth in length of the lamellae of which the shell is made. The figures quoted in column A of Table I show that the degree of arching is independent of the length of the shell. Those given in column C show that the time of onset of the more rapid gryphaeoid type of arching is likewise independent of the length of the shell layers. The figures in columns B and E show a similar independence on the part of the rate of change in expression, and also imply an independence in the time of onset of the phases of expression. The hastening process already mentioned in paragraphs (3) and (4) is exemplified in greater detail by this table. That a similar independence exists for other characters is indicated by the fact that in two specimens of *G. incurva*, in which arching was proceeding at almost the same rate in relation to growth in length, the rate of widening was so different that when arching had attained to 215° the difference in width was 12 mm., that is to say more than half the width of the narrower shell.

Retrogressive characters exhibit the same phenomena. Thus, for example, in *Clavilithes* the transverse ribs recede both in development and evolution as the shell itself increases in calibre.

The combined effect of peculiarities in the behaviour of characters referred to in paragraphs (3) and (4) above have long been recognised by palaeontologists in the use of the phrase "acceleration in inheritance" or "in development" (Cope, 1868; Hyatt, 1874–5, 1889), and by the term tachygenesis. There is, however, a limit to the hastening of the time of onset of characters, for many of them never travel back into the earliest stages of development. There are a few cases known in which after the time of onset of a character has shifted to an early stage of development it experiences delay in later communities. This feature is referred to in the term bradygenesis (Buckman, 1909).

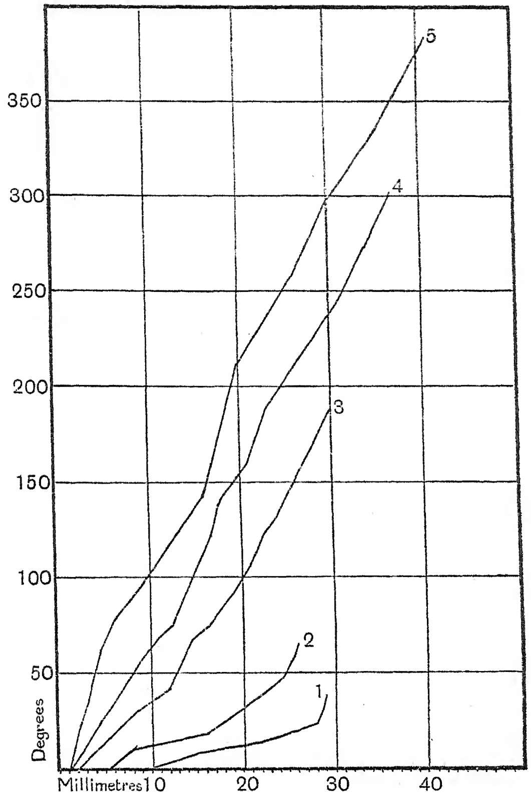

Fig. 4. Graph showing the relationship of arching to growth in size in specimens 1–5 referred to in Table I. Growth in size was obtained by measuring the length of the lamellae, which make up the shell, from hinge line to inferior margin. Arching is expressed by the number of degrees in the angle of rotation between each lamella and the plane of the first formed lamella.

V. THE RELATIONSHIP OF THE PALAEONTOLOGICAL TO THE MENDELIAN UNIT.

Until recently genetic experimentation has been concerned only with characters as they appear in adult life. Much of the material could not in the nature of the case show any other stage because the growth phases were hidden from view either by metamorphosis as in *Drosophila* or by enclosing structures as the pod in peas. Even where growth phases were shown as in the gradual elongation of the internodes of tall peas they do not appear to have attracted attention. This concentration upon the adult phases was no drawback for the study of many of the problems that were being investigated, but it was fundamentally inadequate for the elucidation of questions relating to the origin of characters.

During recent years however, E. B. Ford and J. S. Huxley (1925, 1928), realising this defect, have turned their attention to the developmental as well as the adult phases and by doing so have opened the way to the establishment of a correlation between the findings of palaeontological and experimental workers. The character studied by them was the black pigmentation of the eye in *Gammarus chevreuxi*. Some of the observations on the developmental behaviour of this character upon which they base their factorial interpretations have their counterpart among the features already noted for palaeontological units. They are as follows:

(1) The expression of the character took place so gradually that for the purpose of description it was divided into "fifteen stages." In the first stage, that is at the time of onset, it was almost imperceptible. In the last it was complete.

(2) The time of onset differed in the various lines of descent.

(3) The rate of change of expression also differed.

(4) The pigmentation, the time of onset, the rate of change, all exhibit the outstanding peculiarity of Mendelian units, viz. independent assortment in heredity.

Of these features the existence of (1)–(3) has been proved above for palaeontological units. No. (4) has its counterpart in the independence exhibited not only by the character itself, but also by the time of onset, and by the rate of change when the development of individuals taken from successive communities is compared. In addition the study of the palaeontological units points to the conclusion that the differences noted above, when traced across long ranges of time, are not sporadic, but trend in quite definite directions.

The conjunction of similarities between the developmental behaviour of a Mendelian unit character, and the evolution of a palaeontological unit, creates a strong presumption that the two groups of units are strictly comparable. This is still further strengthened by the fact that conceptions strikingly similar to those arrived at by Ford and Huxley, as the result of their experimental work, had already found a place in palaeontological literature. This may be illustrated by the following quotations. "Each biocharacter has a history of its own, made up of a continuous series of changes moving in a definite direction, from an almost imperceptible or incipient first appearance to a full and extreme expression" (Swinnerton, 1923). After suggesting the term bioseries for such a continuous series of changes the same writer says (1923): "The rate of expression of a single bioseries is by no means constant. During ontogeny it varies, for it may be slow, rapid or even stationary." Another writer says: "There are seriations...of isolated characters, and the transition has not always taken place at the same rate" (Bather, 1920).

Ford and Huxley in discussing the relationship of the thorough genetic analysis carried out on *Drosophila* to their own work say: "If, for example, *Gammarus* were a holometabolous insect, which reached the imaginal stage during the period of maximum divergence of the curves for rapid and slow pigment formation the result would be an animal with two shades of eye colour depending on a single Mendelian factor" (Ford and Huxley, 1928, p. 127).

In summarising their conclusions they say: "It is suggested that the effects of multiple allelomorph series (*e.g.* for eye colour in *Drosophila*) may represent a cross-section through a series of developmental curves of one and the same substance, the curves differing as regards rates of formation of the substance, the times of onset of deposition, and final level of the equilibrium position obtained" (Ford and Huxley, 1925; 1928, p. 133).

These last two statements should be compared with the following extracted from a discussion of palaeontological facts in relation to such units as those investigated in *Drosophila*: "The view adopted here therefore is that the Mendelian unit character is not merely a structural unit, but a structural unit at a certain stage of evolution. The experimenter does not exchange one structural unit for another, but a structural unit at one stage of evolution for a homologous unit at another stage, *e.g.* long staple and short staple in cotton, presence and absence of horns in cattle" (Swinnerton, 1921).

In discussing the above paragraph, F. A. Bather (1927) describes the suggestion it contains as too simple and too unworkable. The work of Ford and Huxley, however, shows that this is not the case.

Such a striking convergence of views is a further indication that both classes of workers are dealing with closely allied phenomena, and that the palaeontological approach is worthy of serious consideration.

Hitherto experimental work has been upon progressive characters. Palaeontologists will await with interest the results of similar work upon retrogressive characters, many of which though present in development find no expression in adult life. *Gammarus* seems likely to provide the requisite material, for Sexton and Clark (1926) have observed: "A new mutation which is dark red when extruded, but light later."

VI. A DISCUSSION OF SOME OUTSTANDING PROBLEMS.

The concentration of attention upon unit characters in fossils opens the way to a fuller understanding of certain questions, some of which have been the subject of much controversy.

(1) *Mutations and transients.*

Granted that the visible palaeontological features discussed above are strictly comparable with those upon which the geneticist bases his factorial studies, the work of Ford and Huxley points a way out of the controversy between the two schools of thought represented by the terms Mutation (De Vries) and Transient; Bather (= Mutation; Waagen (1868)). In the light of their work these terms evidently refer to different phases in the expression of a character. The palaeontologist, with his eye upon developing individuals in successive communities, sees the imperceptible beginnings, and says the character arises as a transient. The average geneticist, with his attention held unconsciously by the adult phase in present-day communities, sees only the full, or nearly full, expression and calls it a mutation.

Work upon retrogressive characters will throw light upon the disappearance of characters, which is almost as important a feature in evolution as the first appearance.

(2) *Independence and allelomorphism.*

In discussing the independence of units two types of character may be recognised, viz. homologous and non-homologous. Homologous characters may be defined as those which occupy the same morphological position in the organism, *e.g.* red and black pigment in the eye, width and narrowness of a shell, smoothness and sulcation of the surface, tallness and shortness in peas. In each of these cases the movement of one character towards full expression necessarily leads to the obscuring or even exclusion of the other. In some cases the allelomorphic pair consists of the same character (*e.g.* stature in peas) in a state of progression (tallness) or of retrogression (dwarfness) respectively. Such characters, which behave as allelomorphs in heredity, seem to appear as differential characters in evolution, and are accordingly of great classificatory value.

Non-homologous characters do not occupy the same morphological position, *e.g.* wrinkled surface in peas and colour of pea flower; width, length and arching in *Gryphaea*. Though such are linked together in combination, each follows its own course in development and evolution. Hybrid crosses of closely allied individuals may lead to allelomorphic exchange, and consequently to the establishment of new combinations. Such exchange does not alter the character itself in heredity. There is no reason to suppose therefore that it would behave differently in either the development or the further evolution of the new combination.

(3) *Combination and linkage.*

No character can exist alone. To find expression it must be in an environment of other characters. The combination thus constituted is an organism. In it the characters are linked together in at least three ways, viz. reverse, parallel and series.

Reverse linkage exists between two characters that are undergoing progression and retrogression respectively. Thus in the evolution of *Gryphaea* the area of attachment retrogresses as the arching progresses.

Characters may be described as in parallel linkage when they are progressing or retrogressing together, though not necessarily at the same rate. In the *Gryphaea* gens arching, widening, transverse convexity are progressing. In *Clavilithes* transverse ribs, roundness of whorl are retrogressing together. Reduplication of the same character, *e.g.* the sulcus in *Inoceramus* and possibly the pigment in the facets of the eye in *Gammarus* may probably be regarded as a special case of parallel linkage.

Serial linkage may be said to exist when one character follows, or is dependent upon, the previous existence of another. Thus in *Inoceramus* there is first the deposition of lime to form the shell, then the appearance of a sulcus which is round in cross-section but which eventually becomes angular. In *Gammarus* black seems to follow red pigment. A special case of serial linkage is that in which as in the case of the septum of *Zaphrentis* progression is followed by retrogression of the same character.

(4) *The theory of recapitulation.*

The wide diversity of opinion upon the validity of the theory of recapitulation is due to an unrealised confusion of two quite distinct phenomena, viz. the behaviour of a unit character on the one hand, and the behaviour of a combination of unit characters on the other. The element of truth, which has preserved the theory from passing into oblivion, is the parallelism which undoubtedly exists between that part of the history of a character which is expressed during the development of the individual, and the fuller record exhibited by the adults in successive communities. The element of error, which has hindered the universal adoption of the theory, is the fact that the phases in the behaviour of one character are not intimately linked with those of another. The most marked divergence arises where two characters are in reverse linkage. Thus in *Gryphaea* the arching of the shell progresses whilst the area of attachment retrogresses. In later communities the latter may disappear completely from development, with the result that the young individual as a combination of characters differs from all early ancestral types. Nevertheless it will resemble those types in some of its characters, as for example in the degree of arching. So then the presence of characters in reverse linkage in any gens will lead to a rapid diminution in the resemblance between developmental stages in later generations and the adults of earlier ones in so far as the combination of these characters is concerned.

With those characters which are in parallel linkage it is to be expected that the resemblance between the whole combination in developmental stages and in adult ancestral individuals will last through a longer or shorter section of the history of the gens according as the rates of change of these characters is nearly the same or widely different. Unfortunately this is not illustrated within the limits of the small number of characters selected for consideration in this paper.

In the case of serial linkage the hastening of the time of appearance of the later character in the series leads to the elimination of the earlier character of the series. Thus the earlier appearance of the sulcus in *Inoceramus* leads to a reduction or even the complete elimination of the smooth surface phase. In *Gryphaea* the hastening of the time of onset of arching has a like effect upon the ostraeoid phase. In *Zaphrentis* the earlier onset of retrogression eliminates the full expression phase of the septum, with the result that the middle of the record is vitiated in the development of the later communities.

Thus the independence of unit characters necessarily leads ultimately to a combination of character phases that had no counterpart in either the young or adult stages of earlier generations. As applied to that combination of characters which makes up the whole organism the error in recapitulation increases with the multiplication of characters and with the length of time that separates the developing individuals which are compared. Herein is the explanation for the fact that the doctrine of recapitulation is on the whole most strongly held by invertebrate palaeontologists, for their material is made up of simpler combinations.

As applied to the individual character the palaeontological evidence in support of this doctrine is overwhelming. But as already pointed out above recapitulation is only one way of describing a series of facts which looked at from another viewpoint may be with equal aptitude described as anticipation. The fundamental phenomenon, however, is neither recapitulation nor anticipation but parallelism, for evolution is not an abstraction, it is a process which proceeds through the medium of developing individuals.

In conclusion a brief reference to the realm of the invisible may be permitted in order to make a suggestion. The "Mendelian factor," which is so stable a feature in heredity that it survives numerous and complicated crossings, must be equally stable in its expression during development and evolution, and consequently its effects should be the same in ontogeny as in phylogeny. Parallelism between those phases of change shown in development and those exhibited in evolution seems indeed to be a corollary of the factorial hypothesis.

VII. SUMMARY.

1. This article is concerned only with visible features, or unit characters, and not with the invisible factors which control their expression.

2. The methods used in establishing a gens are explained, and it is shown that well-established gentes provide suitable material for the study of unit characters.

3. The work of Ford and Huxley upon *Gammarus* furnishes an invaluable link between the investigations of geneticists and those of palaeontologists.

4. For the palaeontologist the study of the behaviour of unit characters in development is fundamental. Peculiarities of such behaviour, long known to palaeontologists, are seen to characterise the development of such a typical Mendelian unit as that investigated by Ford and Huxley. It is inferred therefore that the two classes of units are comparable.

5. This inference is greatly strengthened by the fact that conclusions arrived at by Ford and Huxley concerning the relationship of certain Mendelian units to one another in holometabolous insects had already been independently expressed in palaeontological writings upon the same topic.

6. The correlation of the results of palaeontological and experimental methods of enquiry leads to the discovery of evidence that the terms "mutation" and "transient" merely express different aspects of the behaviour of the same unit.

7. The distinction between a unit character and a combination of unit characters makes it possible to separate the elements of truth and error in the Theory of Recapitulation from one another.

8. It is suggested that parallelism between the behaviour of a character in development and in evolution is a corollary of the factorial hypothesis.

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THE ALL-OR-NONE PRINCIPLE

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I. HEART MUSCLE.

WHILE most organs and tissues behave as a bundle of independent physiological units of which some may be active and others at rest, the heart alone behaves as a single unit under all normal conditions. Simple macroscopic observations of the heart can provide information about a single unit. With other tissues the single units can only be studied by indirect methods or by microscopic observations. For this reason any discussion of the validity of the all-or-none principle should begin with the heart.

The all-or-none character of the beat of the vertebrate heart is too well known to need more than the briefest consideration. Whatever the stimulus, either it is completely ineffective or completely effective, so that the whole organ is excited and responds as completely as possible under the circumstances (Bowditch, 1871). It is necessary to be clear at the outset that the response is not absolutely invariable and that the all-or-none principle merely asserts "that there is an all-or-none *relation* between the stimulus and the propagated disturbance, which it sets up" (Adrian, 1931 *a*). If there are variations in the response they depend upon conditions in the tissue and not upon variations in the stimulus.

Conditions in the heart appear to be as simple as possible as regards the most important variables, the mechanical conditions, certainly simpler than in skeletal muscle (Hill, 1930*b*). Given the conditions for adequate functioning and complete conduction of the excited state, the mechanical response, as measured by the output per beat, is constant except for its variation according to the extent of diastolic filling, that is to say the length of the fibres at the end of diastole (Starling's Law). Moreover, the total energy exchange per beat as measured by oxygen intake varies with diastolic filling but is otherwise constant, as Starling and Visscher (1927) found with dogs' heart-lung preparations, and Clark and White (1928, 1930) with isolated frogs' hearts.

It was of course the very simplicity of the conditions in heart muscle that led to the discovery of the all-or-none character of its response and incidentally to the phenomenon of the refractory phase. But this simplicity has been somewhat misleading, so that the phrase "all-or-none" has been too loosely used. The discussion which follows is intended to make clear the limitation of the principle to the relation between stimulus and propagated disturbance. If another descriptive phrase is needed perhaps it is least misleading to say that every effective stimulus is a threshold stimulus.

II. INSTANCES OF GRADED RESPONSE.

In contrast with the heart muscle, a simple sensory end-organ gives a graded response to a graded stimulus, as the work of Adrian and his collaborators on single nerve fibres has abundantly shown. It will be seen at once that corresponding to this difference the sensory organ has no refractory phase.

The processes in nerve cells or at synaptic junctions appear to be of a continuous and graded character and not all-or-none. At any rate this is the obvious inference from the gradual changes of electrical potential found in nerve ganglia (Adrian and Buytendijk, 1931; and Adrian 1931 *b*). It is worth noting that Sherrington's (1925) theory of central excitation and inhibition implies a graded process. It is not unlikely that secreting cells give a graded response (Mansfeld, Hecht and Kovacs, 1929).

In all these cases there is little doubt that what is graded is a local process, not a propagated one. The local process probably does not liberate and certainly does not transmit any appreciable amount of energy. It may take the form of the temporary disturbance of an equilibrium condition. The local process near a stimulating electrode or at a motor nerve ending is undoubtedly graded, as is seen in changes of threshold for excitation and particularly clearly in the summation of subliminal stimuli. It is only after the local process has exceeded the threshold and set going a propagated disturbance with explosive liberation and transmission of energy that there is an all-or-none process and a refractory phase.

The excitable tissues that remain to be considered are nerve fibres and muscles other than heart muscle. It is clear that each type of tissue must be dealt with separately.

III. THEORETICAL CONSIDERATIONS.

Before dealing with the experimental data the theoretical argument advanced by Adrian (1914) should be considered. If any excitable structure has (1) a definite threshold for excitation, and (2) an absolute refractory period following excitation, its behaviour must be all-or-none provided that (3) the excited state is conducted over the whole structure in question so that it behaves as a single unit. If any one of the three conditions is lacking the response will not be all-or-none. From conditions (1) and (2) it follows that excitation, however brought about, is always liminal in any one place and never greater. The argument is briefly as follows. In order to stimulate, energy must be transferred to the excitable structure (*e.g.* by switching on a current). At some finite time after the process begins the energy will reach the threshold potential at some spot, which will be excited and then immediately become refractory, so that a further increase in potential will not produce any further effect at that spot. As the excitation spreads it will affect in turn every part of the physiological unit. At every point, whether it is excited directly by the external stimulus or by spread of excitation from neighbouring regions, the same thing happens; the effective stimulus is always the threshold stimulus, never greater or less. The argument is independent of the nature of the stimulus, whether it is artificial or the natural stimulus through the nerve endings.

To put the argument inversely, a tissue is refractory because on excitation everything that can happen has happened. If half the total effect could be produced presumably the other half would be immediately producible by a fresh stimulation. Observe that it is essential to the argument that no appreciable time should elapse between the beginning of excitation and the onset of refractoriness.

IV. CHEMICAL ANALOGIES.

A consideration of analogous cases shows that a refractory phase is necessarily connected with an all-or-none process. Explosive chemical reactions have: (1) a threshold, that is they are initiated by a process (a spark) which locally raises the energy potential above a critical minimum value. (2) They have a refractory phase, for once the process is initiated it goes to completion. No further sparking will produce any effect until fresh explosive materials are supplied when a second distinct explosion can be produced. The working of an internal combustion engine or a rifle illustrates perfectly the character of an all-or-none process. So also does any periodical chemical reaction, whether it has spatial periodicity as in the Liesegang phenomenon or a temporal periodicity as in the rhythmic changes that can be obtained under certain conditions when metals dissolve in acids. Hedges (1929*b*) concludes that "the essential condition for periodicity is the existence of some critical condition determining a change which proceeds to completeness once the critical value is reached." A simple case is the electrolytic dissolution of a copper anode in hydrochloric acid (Hedges, 1929*a*). If the current density lies between certain values the metal does not dissolve continuously, but instead a visible film of oxide (or hydroxide) forms on the anode. The resistance at the surface meanwhile rises until a critical potential is reached. Then the oxide dissolves off completely leaving the metal clean. The resistance falls again and of course the local concentration of chlorine ions too. The cuprous oxide formation begins again and the cycle of changes is repeated. Solution of the oxide evidently only occurs when a critical concentration of chlorine ions is reached, then it goes to completion and a fresh film has to be formed again. There is here a conjunction of threshold, refractory phase and an all-or-none process.

This particular reaction is worthy of notice because it is a specially simple case the mechanism of which has been investigated. It is, however, only one among a very large number of similar reactions (see Hedges, 1930, 1931). Of these the phenomena of the "passive" iron wire in nitric acid is too well known to need more than mention (Lillie, 1922, 1925).

In contrast consider an instance of a reversible chemical reaction, where the process is not all-or-none and there is no refractory phase. A small volume of blood or haemoglobin solution is placed in a bottle containing air and a little carbon monoxide. An equilibrium will be established according to the equation:

$$HbCO + O_2 \rightleftharpoons HbO_2 + CO.$$

In the dark the equilibrium position will be right over to the left, all carboxy- and no oxyhaemoglobin. If the blood is exposed to light the equilibrium position is shifted towards the right. Oxyhaemoglobin is formed at a rate varying with the intensity of the light. Under constant illumination the system will settle down to a fresh equilibrium, to be disturbed again by any change in illumination. This model probably provides a close analogy to what happens in the end-organs of the eye, if it is assumed that what excites the nerve is change in concentration of some substance and not the absolute amount or concentration. It probably provides a good analogy of any local process following stimulation which does not liberate much energy and which is not itself propagated, though it may serve to start a propagated disturbance.

V. CONDUCTION WITH DECREMENT.

Dispute as to all-or-none relations has centred round nerve fibres and skeletal muscles. In both there is no doubt about a threshold for excitation and a refractory period, so that the process must initially be all-or-none. The doubt has always been as to the conduction of the excited state. Once excitation has been initiated locally the disturbance can be propagated as long as energy (either of the action current or of some underlying chemical or physical change) is liberated in each segment and transmitted from it so as to reach a potential above the threshold of the neighbouring and still unexcited segment. If potentials and thresholds are similar throughout the unit of tissue, and if the local disturbance is propagated at all, it will be propagated throughout and the response will be all-or-none. But if there is progressive diminution of potential, either because the total energy is less or it is liberated more slowly, or else if there is progressive rise of threshold, then conduction will take place with a decrement. Under the inverse conditions it will take place with an increment. This is "transitional" decrement or increment and is due to a pre-existing gradient in the tissue. It is in no way incompatible with the all-or-none law, for the distance the disturbance travels depends solely upon the pre-existing local conditions in the tissue and not upon the strength of the stimulus. Transitional decrement has been observed in heart muscle under compression (Drury, 1924, 1925), in nerve where there is a gradient of narcosis (Davis, 1926; Davis, Forbes, Brunswick and Hopkins, 1926) and in nerve which is electrically polarised (Bishop and Erlanger, 1926).

What may be called the "classical" conduction with decrement was not of this kind. Lucas (1917) suggested that if the propagated disturbance could vary in size then its size might be measured by its ability to traverse a region of impaired conduction such as a narcotised region. It was assumed that a region of uniform narcotisation could be obtained, for decrement in a transitional region would not produce the required result. It was also assumed that the process in a uniformly narcotised region was graded and therefore, by implication, that there was no refractory period. The assumption of a progressive decrement in uniformly narcotised tissue appeared to be borne out by a number of experiments. Kato (1924, 1926), however, has produced cogent evidence to show that in a uniformly narcotised region, so far as it is experimentally attainable, there is no decrement. The disturbance is evidently smaller and propagated more slowly, but otherwise is the same as in normal nerve. Kato showed that with the very short lengths of nerve that the European workers had used there was inevitably a gradient of narcosis. He also pointed out some other experimental errors by which an apparent decremental conduction had been obtained. (For an account of the whole controversy see Davis, 1926.) The evidence brought forward by Adrian in 1912 to show that the process in normal nerve was all-or-none was based on the assumption that in narcotised nerve it was not all-or-none. As the assumption appears to have been false the experiments are irrelevant, but of course the all-or-none principle must be admitted to hold good. Some later experiments (Adrian, 1914), which do not depend upon any special assumptions as to the character of the process in narcotised nerve, are still relevant to the question. Stimuli which were just maximal were sent into normal nerve above a region which could be narcotised. As narcosis progressed a few fibres just failed to conduct as shown by a diminution in the muscular response. At this stage increasing the strength of the stimulus made no difference, showing that in the least excitable fibres a stimulus above the threshold value was no more effective than a threshold one.

It is very doubtful, however, whether macroscopic experiments on a bundle of independent units can provide a crucial test, which is incapable of being explained away. In particular, experiments based upon the supposed properties of a narcotised region are open to grave doubt. Uniform narcotisation is very difficult to obtain (Davis, Forbes, Brunswick and Hopkins, 1926; Kato *et al.* 1927; Davis and Rice, 1928). Tsai (1931), in experiments on the electrical response in a single fibre with a narcotised region, has found conditions to be very complex, but certainly nothing corresponding to the "classical" conduction with decrement and nothing against the all-or-none law. Consequently recent papers criticising the all-or-none law and based upon macroscopic observations on narcotised nerves can be dismissed without further discussion (Lanczos, 1929; Woronzow, 1931).

To sum up this preliminary discussion: any tissue which is known to be refractory after excitation, as are medullated nerve fibres and vertebrate skeletal muscles, must be considered *prima facie* as behaving in an all-or-none fashion. Experimental evidence against the all-or-none law in these particular tissues must be absolutely unambiguous before it can be accepted. Macroscopic experiments are not likely to produce such evidence. If the evidence turns upon decremental conduction of the excited state, the question must be settled as to whether or not the tissue is refractory after conducting such a disturbance.

VI. ALL-OR-NONE PROCESS IN MEDULLATED NERVE.

The processes in nerve have already been fully discussed (Lucas, 1917; Davis, 1926; Adrian, 1928), so that no detailed consideration is necessary here.

The main evidence that remains to be considered is based upon the observation of a small number of physiological units or of one single one. The first case is the well-known experiment of Lucas (1909) with the frog's dorso-cutaneous muscle. This muscle contains 150–200 fibres and is supplied by a nerve twig with nine or ten fibres, at least one of which is afferent. The number of motor units concerned cannot therefore be more than nine and may be seven or eight. On stimulating the nerve with graded stimuli the response obtained between zero and maximal contraction consisted of four or five distinct steps and the smallest response was half the next smallest. This experiment provided evidence that there is an all-or-none process involved, but could not decide whether it was in the nerve or muscle or both. Recently Kato *et al.* (1931) have carried out a similar experiment, stimulating through one nerve fibre, and have obtained an all-or-none response in the muscle.

Since there is now independent evidence that the processes in the motor nerve fibre is all-or-none, the experiment does not throw any light on the character of the muscular process. But if the stimulus at the nerve ending is of one type only and if each stimulus produces a contraction of the whole muscle fibre, then the muscle fibre under reflex stimulation has no scope for developing a graded response even if it were inherently capable of doing so. Indeed Adrian and Bronk (1929) have shown by direct experiment that the electric response of a single muscle fibre in man and other mammals is all-or-none when reflexly excited. Gradation in reflex muscular response is due to variation in the number of fibres excited and the frequency of excitation. Only by direct stimulation can the all-or-none question be investigated in muscle. Before considering this, however, it will be well to settle the question in nerve.

The beautiful methods developed by Adrian and his collaborators have abundantly demonstrated that when a medullated nerve fibre is stimulated the electric change accompanying the propagated disturbance does not vary in size with the strength of the stimulus. In a sensory nerve increasing the stimulus to the sense organ increases the number of impulses produced and the frequency with which they follow one another. This was first shown by Adrian and Zotterman (1926) by stretching a single muscle spindle in a frog's muscle. Since then similar results have been obtained with many different sensory nerves from different animals (*e.g.* Adrian and Umrath, 1930; Matthews, 1931; Adrian, Cattell and Hoagland, 1931; summary of earlier work, Adrian, 1928). The impulses passing down motor nerves from the central nervous system are also similar (Adrian and Bronk, 1928, 1929).

The only possible doubt is whether the size of the electric response in nerve is a reliable index of the size of the propagated disturbance. This question has been much discussed (see Davis, 1926), but can hardly be answered except in the affirmative. The electric change occurs when and where the nerve is active. An impulse in narcotised nerve or partially refractory nerve may be expected on other grounds to dispose of less energy and gives a correspondingly smaller electric response. Where, in comparing different nerves, the electric response shows specific differences they correspond with other functional differences (Matthews, 1929; Erlanger and Gasser, 1924). Lastly it has been shown in frog's heart that there is an extremely close relationship between the electrical and mechanical responses even if they are not completely inseparable (Einthoven and Hugenholtz, 1921; Arbeiter, 1921). In frog's skeletal muscle Roos (1932) concludes that the electrical and mechanical response begin simultaneously. At any rate the electric change is nothing incidental or casually related to other processes, but is an important part of the propagated disturbance. It is unlikely that the propagated disturbance could alter in any significant way without a corresponding change in the electric response. If for any reason the electric response were to be dismissed as no true criterion of the size of the propagated disturbance in nerve, there would simply be no criterion left. In conclusion there seems to be no valid reason to doubt that the process in the medullated nerve fibres is all-or-none.

VII. NON-MEDULLATED NERVE.

Recent work on the action potentials of individual non-medullated nerve fibres in mammals and amphibians confirms the earlier observations in suggesting that there is no fundamental difference between the processes in them and in medullated nerve. The rate of conduction of the disturbance is slower and the electric change lasts for a longer time, but there is nothing to suggest the absence of a refractory phase or any departure from the all-or-none law (Erlanger and Gasser, 1930; Adrian, Cattell and Hoagland, 1931; Adrian, Bronk and Phillips, 1932).

The same may be said about the recent work on crustacean nerves. The striking thing about them is the slowness of the recovery process and the large amount of energy expended in it (Levin, 1927; Furusawa, 1929; Hill, 1930*a*). There is no indication of absence of refractory phase or gradation of response. It is true that with frequent stimulation the electric response diminishes in size, but that is simply because of the long time taken for recovery; the impulses are passing along incompletely recovered tissue. The observation of Barnes (1930, 1932) that a prolonged series of impulses uniform in size and rhythm come from an injured end of nerve also indicates the all-or-none character of the process.

Jordan (1928) has suggested that there may be conduction with decrement in crustacean nerve, but not, it would appear, on the basis of any definite data. He has also suggested that in the primitive nerve net of such animals as coelenterates there may be conduction with decrement. This suggestion may very well be correct and is in no way incompatible with an all-or-none process in individual fibres. The decrement may very well be transitional decrement. Some such decrement must occur in the musculature of the mammalian gut when a peristaltic wave after travelling some distance dies out. But as the excitation has to pass from cell to cell (whether in the nerve plexus or in the muscle cells) it is only natural that under certain conditions successive cells should be less able to be excited or to transmit to others.

VIII. SKELETAL MUSCLE.

Considerable difficulties arise in interpreting the evidence from skeletal muscle. Not only do individual muscle fibres respond independently, but their response is evidently a complex sequence of events liable to considerable variation according to the conditions in the fibres. It must be remembered too that the summation of contractions following a series of stimuli is a graded process. The most important evidence has been obtained by recording the response of a single muscle fibre stimulated directly by an induction shock from a very small electrode. The necessary technique has been worked out by Pratt (1930).

The method used was to stimulate one or a few fibres on the surface of a frog's sartorius muscle by an electrode consisting of a small capillary opening in a glass tube (the other electrode was a diffuse one). Minute drops of mercury were scattered on the surface and the excursion of one of them could be observed under the microscope and photographically recorded at the same time. The extent of displacement of the drop indicated the extent of the response of the individual fibre on which it rested and it was possible to distinguish a response of that particular fibre from passive movements due to neighbouring fibres. In the earlier experiments the pores in the electrodes were not the very smallest ($> 25\mu$ diameter), and the micro-electrode was at some distance from the part of the fibre under observation. Under these conditions a graded stimulus did not produce a graded response of the fibre, but the contraction was all-or-none (Pratt and Eisenberger, 1919).

Fischl and Hahn (1928) reopened the question as the result of observations on the retro-lingual membrane of the frog, where there are scattered fibres of striated muscle. Using large electrodes these authors found what they considered was a graded response in a single fibre. Hintner (1930) criticised their technique and concluded that a graded response was only obtained in damaged fibres, owing to incomplete conduction. Pratt (1930) also criticised their technique and failed to confirm their results. He claimed that with any type of electrode but the very smallest the response was always all-or-none; but he admitted that under certain special conditions something like a graded response could be obtained.

The matter has been further investigated by Gelfan (1930) and Gelfan and Gerard (1930) using the retro-lingual membrane. They used electrodes only a few microns in diameter, either capillaries or fine wire insulated except at the tip, and applied them to an individual fibre close to the point of observation. Then with induction shocks near the threshold value a localised contraction was obtained which varied with the strength of stimulus. With a stronger stimulus a greater length of fibre contracted. This localised contraction might extend as far as 1 mm. from the electrode, but beyond that with stronger shocks the excitation would spread over the whole fibre and the ordinary all-or-none response was obtained. If the electrode was a little distance from the fibre or was not so small, the localised contraction was not obtained. Similar localised contraction with small electrodes and weak stimuli have been observed in curarised frog's sartorius muscles (Gelfan, 1931).

Gelfan and Gerard (1930) explain the phenomena of localised contraction on the assumption that it is the sarcolemma that conducts the excitation over the whole fibre to the individual sarcomeres which do not necessarily transmit the excitation themselves. The localised effect therefore is due to the fact that if the electrodes are close enough and small enough, individual sarcomeres may be directly acted on by the current without it affecting the sarcolemma, a fairly large area of which has to be traversed by a current of threshold intensity before it is excited. If this is correct it is easy to understand how injury to a muscle fibre by damaging the sarcolemma favours the production of a localised response (Hintner, 1930); and it accounts for the finding of Asmussen (1931) who dissected out single fibres, an operation hardly possible without injury, and obtained graded contractions.

The small localised contraction observed by these authors does not appear to differ from the local contraction at the cathode which can be seen with weak constant currents and which has been known for a very long time. With increasing strength of current the effect extends farther from the electrodes (Biedermann, 1895, pp. 176 foll.). Lucas (1908) observed that it occurs with currents of about threshold intensity, and from the figure in his paper (p. 466) it can be inferred that it has a latent period and initial speed of onset of the same order as a small twitch. With an induction shock therefore it would take the form of a localised twitch only distinguishable from an ordinary twitch by the absence of conduction of the active state. If one electrode is small and the other diffuse, whichever way the current runs there will be an effective cathode at a region in the fibre close to the small electrode.

It is not clear what the nature of the local cathodic contraction is. Is it a passive deformation of an elastic structure produced by polarisation, or is there a localised liberation of energy? And if so, the liberation of energy can hardly follow the same course as that in a normal contraction, as there is no refractory phase. The fact that all excitable tissues subjected to a constant stimulus become "adapted" (Adrian, 1928) after a time and cease to respond, suggests that this effect of a constant current which apparently continues as long as the current continues is not a process of excitation in any proper sense of the word. That is to say, it perhaps does not involve any liberation of energy by the tissue. In favour of this Biedermann (1895) quotes an old observation of Schiff who found that a moribund muscle which no longer gave a twitch when a constant current was made still gave a local cathodic contraction.

Whether or not the local effect observed by the American workers using microelectrodes is the same as the local cathodic contraction, it seems clear from their results that provided the excitation is propagated along the fibre its response is all-or-none.

It is not safe to assume that the only effect of sending an electric current through a muscle is to produce excitation, and that if a fibre is not excited nothing happens to it, as the above discussion suggests. A much more striking effect, however, is that described recently by Stewart (1932) as the result of analysis of graphic records of the frog's heart beat. He finds that a stimulus applied to the relaxing sinus may have no effect at all in the sense of causing the next sinus beat to begin sooner, but nevertheless may result in altered character of the contraction (quicker relaxation) when it does occur. That is to say although the tissue is completely refractory in the sense that it is not excited it is altered in some way by the current sent in.

Hartree and Hill (1921) argued that if the contractile process is all-or-none, then in a single twitch of a parallel-fibred muscle like the frog's sartorius, varying the stimulus should lead simply to an alteration in the number of fibres contracting and should leave unchanged the ratio of heat liberated to tension developed. This they did not find, but the ratio H/T varied in a complicated way with the strength of stimulus, and they infer a partial failure of the all-or-none law. But as the variations in stimulus involved sending induction shocks of varying strength through all the fibres, the fibres were not necessarily in the same condition in each case. There is a further objection to the argument. With a submaximal stimulus, the active fibres, unless they are uniformly distributed throughout the length of the muscle, will stretch the inactive ones, and the twitch will not be strictly isometric. The fact that the relaxation curves from maximal and submaximal twitches are not superposable gives support to this suggestion. Further, even when a twitch is maximal so that all fibres contract something of the same sort may happen if the excitation starts near one end of the fibres and spreads along to the other. The rate of conduction is sufficiently slow relative to the latent period for the active end to effect some stretching of the end not yet active. It may be suggested that possibly the reason why Hartree and Hill found that with a supramaximal stimulus the ratio H/T was often smaller than with one just maximal was that the stronger stimulus produced a more nearly synchronous contraction along the whole length of the fibres.

At any rate these arguments serve to show that in any macroscopic experiment apparent exceptions to the all-or-none law can always be explained away, and only microscopic observations can provide unequivocal evidence.

IX. VERTEBRATE UNSTRIATED MUSCLE.

The anatomical arrangement of most smooth muscles makes investigation difficult along the lines that have been successful with skeletal muscle. The fibres are generally spread out in thin sheets along with much non-muscular tissue and often with nerve cells. Fine fibrils sometimes connect different fibres, but it is difficult to say how complete or incomplete the functional connection may be.

The behaviour on stimulation adds to the difficulties. In order to establish the existence of a refractory period and to obtain clear evidence for or against all-or-none behaviour, it is essential to be able to get a definite and reproducible response with a single instantaneous stimulus. Though smooth muscle responds well to a series of induction shocks or to constant current flowing for times of the order of a second, usually instantaneous electric stimuli (*i.e.* of the order of a few thousandths of a second) only excite a few of the most excitable fibres. When a stimulus is spread out over an appreciable time the response may in part at least be the result of summation of contractions. Whether or not each individual response is all-or-none the result of summing them may be graded. It is difficult too to be certain by means of a prolonged stimulus whether or not a tissue is refractory after excitation. These arguments apply with even greater force to the action of drugs, which are most efficient stimuli but whose action is of considerable duration.

It has been found in many cases that if a curve is plotted relating the amount of a stimulating drug present and its effect on the organ, the curve is **S**-shaped (see Trevan, 1927). It has been suggested by Fromherz (1926), who studied the effect of extracts of pituitary posterior lobe in stimulating the uterus, that the curve was a frequency curve and that the contraction represented the statistical result of all-or-none contractions of fibres with different thresholds. All that can really be concluded is that the observed facts are compatible with all-or-none contractions of individual fibres, but they are equally compatible with graded contractions.

If it could be established that they were myogenic, the spontaneous rhythmic contractions found in parts of the musculature of the gut, *e.g.* the pyloric region of the stomach, would be strong evidence in favour of an all-or-none contraction and a refractory period. It is true that the contractions are irregular and vary in size, but this can be accounted for by incomplete synchronisation of different groups of fibres. Unfortunately, though the balance of evidence seems to be in favour of a myogenic origin, it is not conclusive (C. J. Hill, 1927).

Alvarez (1917), working with the rhythmically contracting frog's stomach, found that by stimulation an extra contraction could be superposed upon any part of a spontaneous wave but that there was some degree of refractoriness and a lengthened latent period. If there is any absolute refractory period in this muscle it must be very short.

On the other hand Schüller (1921) found a refractory period in the rhythmically contracting frog's rectum. An extra stimulus sent in up to the time of the peak of contraction was ineffectual, but it was effectual on the relaxing muscle. Prolonged tetanic stimulation tended to produce very incomplete fusion of contractions. So that in this organ at any rate there is a refractory period. There appears to be a distinct tendency for the whole isolated rectum to behave in an all-or-none way; that is to say, for the rhythmic spontaneous contractions to be maximal and for an interpolated stimulus if effective at all to produce a maximal response.

The prolonged "tonic" contraction found in many types of smooth muscle might seem a stumbling-block in the way of assuming an all-or-none process, but it is possible that "tone" in smooth muscle is like "tone" in skeletal muscle and is essentially a tetanus produced by asynchronous activity in different groups of fibres (Ritchie, 1928, p. 78). If this were so the underlying process in the fibres might be all-or-none. But this is not more than a possibility.

It must be admitted that although the processes in some kinds of unstriated muscle are very probably all-or-none, there may be others where they are not.

X. INVERTEBRATE MUSCLE.

The investigation of the slower types of invertebrate muscle is attended by difficulties similar to those met with in vertebrate smooth muscle. But there are some relevant observations, chiefly on the quicker types of muscle.

Bozler (1927) was able to stimulate single fibres of the body muscles of the ctenophore *Beroë*, and to observe the contraction under the microscope. The response to varying stimuli was found to be all-or-none. Similar observations were made on the chromatophore muscles of cephalopods (Bozler, 1928, 1931). The chromatophores are expanded by the contraction of a single ring of radial muscle fibres. In a piece of isolated skin the muscles are at first usually relaxed. A single induction shock produces a twitch and momentary expansion of the chromatophores, which can be photographically recorded (Bozler, 1931). These twitches are found to be all-or-none.

The phenomena are complicated by the fact that the chromatophores may remain expanded for long periods. This "tonic" contraction of the muscles can hardly be the result of a fusion of twitches, because a twitch can be superposed on it. The state of "tone" may be diminished by stimulation, and this inhibition is graded according to the strength of the stimulus. Whatever the real character of the state of tone may be, as it is a process extended over a considerable period of time it probably resembles a tetanus at least to this extent, that it is the result of summing a series of processes of shorter duration. The "tonic" process cannot therefore from the nature of the case be all-or-none, and it is not very likely that the process of abolishing it should be either. In any case no process with a refractory phase appears to be involved.

The retractor muscle of *Sipunculus*, which is a slow muscle, was found by Fuchs (1910) to have a refractory period. If stimuli were sent in in rapid succession there was only a single electric response corresponding to the first, whereas with a slow succession each one produced a separate electric and mechanical response. The phenomena here are those of "Wedensky inhibition" and are complex, but do indicate clearly a refractory phase.

The quick (striated) adductor muscle of *Pecten* produces rhythmic contractions under its normal reflex stimulus. A single induction shock is an effective stimulus, but a rapid series (faradic stimulation) produces incomplete fusion of contractions (Bayliss, Boyland and Ritchie, 1930). This strongly suggests that there is a refractory period lasting till about the peak of contraction.

There are therefore indications that the contractile process in many invertebrate muscles is all-or-none. But the possibility of others that do not behave in the same way can hardly be ruled out, seeing how few have been investigated.

XI. SUMMARY.

1. The all-or-none character of the heart beat is briefly considered, and, in contrast, some processes which are graded. A theoretical discussion follows emphasising the relation between an all-or-none process and a refractory phase following excitation. Some chemical analogies are mentioned.

2. The difficulties surrounding the question of conduction with a decrement are discussed and the necessity for observations on single physiological units.

3. The all-or-none character of the nervous impulse, which the older macroscopic observations strongly supported, is now placed beyond doubt by the investigation of action potentials in single nerve fibres.

4. The case of skeletal muscle is not so easily settled, but the conclusion appears to be that its contractile process is all-or-none. Apparent exceptions and anomalies are discussed.

5. Among vertebrate smooth muscles and various invertebrate muscles there is evidence of all-or-none behaviour in certain types at least. But it would be premature to conclude that they are all similar.

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LA SIGNIFICATION BIOLOGIQUE DES POTENTIELS D'OXYDORÉDUCTION

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(With Six Text-figures.)

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AU cours des dix dernières années, de nombreux travaux ont eu pour objet l'étude des potentiels d'oxydoréduction des milieux biologiques. La signification physiologique de ces recherches n'a pas toujours été clairement exprimée; la raison en est, comme nous le verrons au cours de cet exposé, que ces mesures sont particulièrement importantes dans un domaine de la physiologie qui n'est pas le plus communément travaillé. Avant de nous placer à ce point de vue, il convient toutefois d'insister sur les services que peut rendre à la biologie en général la détermination des potentiels d'oxydoréduction, indépendamment de toute théorie proprement physiologique.

I. DÉTERMINATION DE L'ÉNERGIE LIBRE DE CERTAINES RÉACTIONS D'OXYDATION.

1. *Principe de la détermination de l'énergie libre.*

La détermination des énergies libres des réactions s'impose chaque fois que l'on envisage des rapports entre différentes réactions, c'est à dire chaque fois que l'on considère l'aspect chimique des faits physiologiques. On sait en effet que, d'après le deuxième principe de la thermodynamique, une réaction ne peut se faire spontanément, c'est à dire sans qu'on lui fournisse de l'énergie extérieure, que si elle est capable d'effectuer du travail quand elle se poursuit dans les conditions de réversibilité et à température constante. Le travail effectué dans ces conditions représente le travail maximum w que l'on peut admettre de cette transformation spontanée, et on lui donne par convention le signe positif.

Comme la plupart des réactions se font à la pression atmosphérique, le travail réellement utilisable est ce travail maximum diminué du travail effectué contre la pression constante de l'atmosphère P. On appelle cette fonction la variation d'énergie libre ΔF de la transformation et on lui donne le signe inverse du travail

$$-\Delta F = w - P\Delta V \qquad \text{......(1)},$$

ΔV étant la diminution de volume qui accompagne la réaction.

D'après le deuxième principe, une réaction chimique ne peut donc évoluer spontanément à pression et température constantes que si elle correspond à une variation d'énergie libre négative, c'est à dire si l'on a $\Delta F < 0$. Il y a équilibre pour $\Delta F = 0$. Ainsi, selon que l'énergie libre d'une réaction est négative ou positive dans les conditions où elle s'effectue, il convient de la considérer sans lien nécessaire avec une autre, ou, au contraire, de rechercher les autres réactions couplées avec elle. Les problèmes soulevés par la contraction musculaire, l'action dynamique spécifique, le mécanisme des synthèses, pourraient être largement éclaircis si l'on disposait de données quantitatives sur l'énergétique des réactions impliquées. Nous disposons de plusieurs méthodes pour déterminer leur énergie libre:

1°. *L'application du principe de Nernst.* D'après le premier principe de la thermodynamique, l'accroissement d'énergie interne d'un système est égal à la chaleur *absorbée* q, diminuée du travail w *effectué* par le système:

$$\Delta U = q - w \qquad \text{......(2)}.$$

Quand une réaction se fait réversiblement à température et à pression constantes, on a, d'après les relations (1) et (2):

$$\Delta F = \Delta H - q,$$

si l'on désigne par ΔH la chaleur de réaction à pression constante, c'est à dire la chaleur dégagée ou absorbée par la réaction quand aucun travail extérieur autre que celui de l'atmosphère n'est effectué. On trouve généralement sa valeur dans les tables thermochimiques.

Quant à q, qui est la chaleur de réaction à pression constante *dans les conditions de réversibilité*, c'est à dire quand la réaction effectue son travail maximum, on peut calculer sa valeur de la manière suivante:

q est la différence entre l'énergie calorifique qu'il faut fournir aux corps initiaux de la réaction pour les amener, en partant du zéro absolu, dans les conditions de température et de concentration expérimentales, et celle qu'il faut fournir aux corps finaux de la réaction pour les amener, en partant également du zéro absolu, dans ces mêmes conditions de température et de concentration expérimentales, toutes ces opérations étant effectuées de manière réversible et à pression constante. Pour chacun des corps en question cette énergie calorifique est égale au produit par la température absolue T de ce que l'on appelle l'entropie de ce corps. On peut calculer cette entropie, pour chaque corps, si l'on connaît ses chaleurs spécifiques depuis le zéro absolu jusqu'à la température T, ainsi que les chaleurs des diverses transformations qu'il subit dans cet intervalle de température, et à condition d'admettre l'hypothèse de Nernst, d'après laquelle l'entropie de tous les corps purs est nulle au zéro absolu. Si l'on appelle ΔS la différence entre la somme des entropies des corps initiaux et la somme des entropies des corps résultant de la réaction, on a donc:

$$\Delta F = \Delta H - T\Delta S \qquad \ldots\ldots(3).$$

Le manque de données sur les chaleurs spécifiques, aux basses températures, des corps intervenant dans les réactions biologiques rend cette méthode encore rarement applicable, malgré le travail considérable effectué par l'école de G. N. Lewis.

2°. *Le calcul du travail effectué par la réaction dans les conditions de réversibilité.* Dans la méthode précédente on obtenait par voie indirecte la variation d'énergie libre, à partir de simples données thermiques. Deux autres méthodes fournissent directement ΔF. Dans la première de celles-ci on calcule le travail maximum qui correspond à la réaction chimique. Considérons la réaction entre des corps que nous supposerons d'abord être des gaz parfaits:

$$\mathrm{aA} + \mathrm{bB} \rightarrow \mathrm{a'A'} + \mathrm{b'B'} \qquad \ldots\ldots(\mathrm{I}).$$

Le travail maximum de cette réaction correspond à la somme des travaux suivants: on fait passer les a molécules de A de leur pression dans les conditions de l'expérience à une pression pour laquelle A est en équilibre avec B, A' et B'. Nous faisons de même pour les b molécules de B. On effectue la réaction à pression constante, ce qui ne correspond à aucune variation d'énergie libre puisque A et B sont maintenant en équilibre avec A' et B'. Nous faisons ensuite passer les a' molécules de A' et les b' molécules de B' qui se sont formées, de leur pression d'équilibre à la pression où ces corps se trouvent à la fin de la réaction. La variation d'énergie libre qui correspond au passage d'une molécule gramme d'un gaz parfait de la pression p_1 à la pression p_2 est:

$$\Delta F = RT\, ln \frac{p_2}{p_1} \qquad \ldots\ldots(4),$$

R étant la constante des gaz. Pour connaître l'énergie libre de la réaction (I), il suffira donc d'additionner les variations d'énergie libre, calculées d'après l'équation (4), correspondant aux opérations que nous avons effectuées avec les molécules A, B, A' et B'. Pour que les calculs restent simples, quand on n'a pas affaire à des gaz parfaits, nous emploierons l'artifice de G. N. Lewis qui consiste à substituer à la pression une grandeur que l'on appelle l'*activité* et qui est précisément définie de telle sorte que la relation (4) soit encore exacte, une fois opérée cette substitution. On peut alors calculer l'énergie libre de la réaction (I).

Désignant par [A] et [B] les activités des corps initiaux A et B et par [A'], [B'] les activités des corps finaux A' et B', et en désignant par l'indice *e* les activités de A, B, A', B' à l'état d'équilibre, l'énergie libre de la réaction est:

$$\Delta F = RT\, ln \frac{[\mathrm{A'}]^{a'}.[\mathrm{B'}]^{b'}}{[\mathrm{A}]^{a}.[\mathrm{B}]^{b}} - RT\, ln \frac{[\mathrm{A'}]_e^{a'}.[\mathrm{B'}]_e^{b'}}{[\mathrm{A}]_e^{a}.[\mathrm{B}]_e^{b}} \qquad \ldots\ldots(5),$$

ou $$\Delta F = RT \ln \frac{[A']^{a'} . [B']^{b'}}{[A]^{a} . [B]^{b}} - RT \ln K,$$

K étant la constante d'équilibre de la réaction (I).

Si donc on peut réaliser, au moyen d'un catalyseur convenable, l'équilibre entre A, B, A′ et B′, et obtenir par analyse les concentrations de ces substances à l'état d'équilibre, si enfin on peut calculer à partir de ces concentrations les valeurs des activités, ou admettre que celles-ci sont égales aux concentrations, on connaîtra K et par conséquent on sera en mesure de calculer ΔF pour toutes les concentrations de A, B, A′ et B′.

3°. *La mesure directe du travail effectué par la réaction dans les conditions de réversibilité.* Les réactions d'oxydation et de réduction se prêtent spécialement à ces mesures parce qu'elles sont susceptibles de fournir du travail électrique.

En effet, l'on comprend ordinairement sous le terme d'oxydation, d'une part l'oxygénation, c'est à dire la fixation d'oxygène, comme la combustion du carbone:

$$C + O_2 \rightarrow CO_2$$

et la déshydrogénation comme la transformation de l'éthane en éthylène:

$$C_2H_6 \rightarrow C_2H_4 + H_2,$$

d'autre part, des réactions où peuvent n'intervenir ni oxygène, ni hydrogène et qui sont en réalité des changements de valence. Telle est l'oxydation par perte de charge négative d'un ion, par exemple d'un ion ferreux devenant ferrique:

$$Fe^{++} \rightarrow Fe^{+++} + e,$$

ou d'un élément électropositif, par exemple l'hydrogène, qui s'ionise:

$$H_2 \rightarrow 2H^+ + 2e \qquad \text{......(II).}$$

Dans ces deux dernières équations e représente un électron. Considérons par exemple la dernière réaction quand elle est à l'équilibre. On peut traiter l'électron comme une molécule d'un gaz parfait et définir l'activité électronique du système de la manière suivante:

$$[e] = \sqrt{K \frac{[H_2]}{[H^+]^2}} \qquad \text{......(6).}$$

L'ionisation de l'hydrogène tend à accroître l'activité électronique du milieu.

On désigne sous le nom de réductions les réactions inverses des précédentes, c'est à dire des désoxygénations, des hydrogénations, des pertes de valence, l'ionisation d'un élément électronégatif tel que l'oxygène:

$$\tfrac{1}{4}O_2 + \tfrac{1}{2}H_2O + e \rightarrow OH^-.$$

La réunion sous un même terme de ces différents types de réactions est thermodynamiquement logique puisque appauvrir un milieu en oxygène ou l'enrichir en hydrogène revient potentiellement à accroître son activité électronique. Au moyen d'un catalyseur convenable, on peut toujours rendre cet accroissement électriquement mesurable.

Quoi qu'il en soit, lorsque l'oxydation consiste effectivement dans une variation de charge, cette charge peut être dérivée sur une électrode inerte et s'en aller neutraliser un autre ion au contact d'une deuxième électrode.

Le travail électrique correspondant à ce transport a pour expression le produit de la force électromotrice par le nombre $n\mathfrak{F}$ de coulombs transportés, n étant le nombre d'équivalents impliqués dans la réaction, et $\mathfrak{F}$ le Faraday, soit 96494 coulombs. Ce travail mesure l'énergie libre de la réaction totale effectuée si la transformation se fait dans les conditions de réversibilité. La force électromotrice est, dans ce cas, égale à la différence de potentiel E entre les deux électrodes. Nous écrirons:

$$E = \frac{\Delta F}{n\mathfrak{F}} \qquad \text{......(7).}$$

On pourrait tout aussi bien écrire $E = -\dfrac{\Delta F}{n\mathfrak{F}}$, ce que font de nombreux auteurs.

En raisonnant sur les électrons comme on fait pour un gaz parfait, c'est à dire en considérant l'activité électronique telle qu'elle a été définie dans la relation (6), l'énergie libre de ce transport correspond au travail nécessaire pour amener réversiblement le nombre d'électrons correspondant à 96494 coulombs d'un milieu dont l'activité électronique est $[e]$ dans un milieu où cette activité est $[e']$. Cette énergie libre a donc comme expression pour un Faraday:

$$\Delta F = RT\,ln\,\frac{[e']}{[e]}.$$

Considérons deux solutions dans chacune desquelles on a plongé une électrode inerte, c'est à dire constituée par un métal qui n'émet pas d'ions dans la solution. La différence de potentiel entre les deux électrodes ne dépend que des activités électroniques $[e]$ et $[e']$ des deux solutions, et permet de mesurer l'énergie libre de la réaction qui s'effectue entre elles quand un Faraday est échangé:

$$E = \frac{RT}{\mathfrak{F}}\,ln\,[e'] - \frac{RT}{\mathfrak{F}}\,ln\,[e] \qquad \text{......(8).}$$

Nous ne connaissons jusqu'ici qu'une différence de potentiel entre deux milieux, c'est à dire que nous n'atteignons que des différences d'énergie libre. On peut choisir une solution de référence dont arbitrairement nous poserons que l'activité électronique $[e']$ est égale à l'unité. Par convention, ce sera l'activité électronique d'un système standard constitué par de l'hydrogène moléculaire à la pression de 1 atmosphère et une solution d'ions hydrogène d'activité égale à l'unité, c'est à dire le système ($[H_2] = [H^+] = 1$). Cela revient à fixer à zéro la valeur de la différence de potentiel entre une électrode de platine platiné[1] et une solution acide saturée d'hydrogène gazeux à 1 atmosphère et dont l'activité en ions hydrogène est égale à 1. D'après la convention de signe que nous avons choisie pour la relation entre ΔF et E, la différence de potentiel entre cette électrode, que l'on appelle l'électrode normale d'hydrogène, et une électrode inerte plongée dans une solution quelconque sera positive si la solution tend à oxyder le système ($[H_2] = [H^+] = 1$)

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[1] Le dépôt platiné catalyse l'équilibre entre les ions H^+ et l'hydrogène.

et négative si elle tend à le réduire. Dans la suite, on désignera par E_h la différence de potentiel d'un système par rapport à l'électrode normale d'hydrogène. L'équation (8) devient:

$$E_h = -\frac{RT}{\mathfrak{F}} \ln [e] \qquad \text{......(9).}$$

Les valeurs de E_h ainsi mesurées permettent donc de classer les divers systèmes chimiques suivant leur possibilité de s'oxyder ou de se réduire mutuellement, et de calculer l'énergie libre correspondant à leur oxydation ou leur réduction par le système de référence.

Dans la pratique, on substitue au système de référence ($[H_2] = [H^+] = 1$) d'autres systèmes étalons tels que celui qui est constitué par une électrode de calomel, et dont le potentiel par rapport au système de référence a été soigneusement établi.

2. *Détermination de l'énergie libre d'oxydoréduction des corps non électroactifs.*

Les réactions qui intéressent le plus les biochimistes se font rarement entre des corps qui agissent directement sur les électrodes. On peut cependant effectuer une détermination de leur énergie libre d'oxydoréduction quand ces corps peuvent réagir avec des substances qui échangent elles-mêmes des charges avec les électrodes et que l'on peut appeler *électroactives*. Considérons une de ces réactions. Ce sera, par exemple, l'oxydation de l'acide succinique en acide fumarique; en présence d'une diastase, l'acide succinique cède de l'hydrogène au bleu de méthylène.

L'expérience montre que la réaction est une réaction d'équilibre. Cette réaction peut être décomposée en:

$$\text{acide succinique} \rightleftharpoons \text{acide fumarique} + H_2$$

$$\text{bleu de méthylène} + H_2 \rightleftharpoons \text{leucobase du bleu de méthylène.}$$

Nous cherchons à déterminer l'énergie libre correspondant à la première de ces réactions, que nous écrirons:

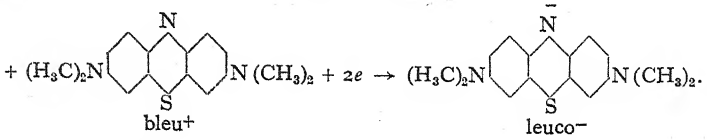
$$\text{succinate}^{=} \rightarrow \text{fumarate}^{=} + H_2 \qquad \text{......(III).}$$

Quand les trois constituants de cette réaction sont pris à l'activité 1, cette énergie libre est égale à $\Delta F = - RT \ln K$ où K est la constante d'équilibre

$$K = \frac{[\text{fumarate}^{=}]}{[\text{succinate}^{=}]} [H_2].$$

On calcule aisément cette constante. On a mis en présence de la diastase une certaine quantité de succinate, de fumarate et de bleu de méthylène. Une simple mesure colorimétrique indique la concentration du colorant non réduit, une fois l'équilibre atteint, et l'on en déduit le rapport $[\text{succinate}^{=}]/[\text{fumarate}^{=}]$ à ce moment. Il ne reste donc à connaître, pour déterminer la valeur de K, que l'activité de l'hydrogène.

Or, le bleu de méthylène est en équilibre électrochimique avec son produit de réduction comme l'ont montré M. Clark et ses collaborateurs (1925). Le bleu de méthylène est un colorant basique; c'est même une base forte qui reste dissociée en solution très alcaline. Il émet en solution des cations que nous désignerons par bleu⁺ pour marquer qu'ils portent une charge positive. Ces ions peuvent être réduits en captant deux électrons qui les transforment en un anion que nous désignerons par leuco⁻. Le schéma ci-dessous représente la réaction:



Ainsi, par l'intermédiaire du bleu de méthylène, l'hydrogène de l'acide succinique est mis en équilibre électrochimique, si bien que l'on peut écrire, d'après les équations (6) et (9), que le potentiel du mélange est:

$$E_h = \frac{RT}{\mathfrak{F}}\, ln\, [H^+] - \frac{RT}{2\mathfrak{F}}\, ln\, [H_2].$$

En posant:

$$pH = \log \frac{1}{[H^+]} \quad \text{et} \quad rH_2 = \log \frac{1}{[H_2]}$$

on obtient:

$$E_h = -\frac{1}{0{\cdot}4343}\frac{RT}{\mathfrak{F}}\, pH + \frac{1}{0{\cdot}4343}\frac{RT}{2\mathfrak{F}}\, rH_2 \qquad \text{......(10)},$$

le facteur $\frac{1}{0{\cdot}4343}$ s'introduisant par la transformation de $ln\,[H^+]$ et $ln\,[H_2]$ en logarithmes à base 10.

Cette équation permet quand on connaît l'activité de l'hydrogène, par exemple quand on mesure le potentiel d'une électrode de platine platiné saturé d'hydrogène à 1 atmosphère, ce qui correspond à $rH_2 = 0$, de déterminer l'activité en ions H^+ d'une solution. Inversement, quand on connaît cette activité en ions H^+, quand on opère dans une solution tampon mesurée avec l'électrode d'hydrogène avant l'addition du système oxydoréducteur, on peut déterminer électrométriquement l'activité de l'hydrogène. On trouve, dans le cas de l'acide succinique, que, si le rapport [fumarate⁼]/[succinate⁼] est égal à 1, le potentiel d'équilibre du mélange avec le bleu de méthylène, d'après les données recalculées de Quastel et Whetham, est, à 45° C. et à pH 7·2, égal à $-0{\cdot}030$ volt. On a donc, d'après l'équation (10) dans laquelle $R = 8{\cdot}316$ joules/degré, $T = 318$, $rH_2 = 13{\cdot}4$. La constante d'équilibre K de la réaction (III) est donc égale à $10^{-13{\cdot}4}$, et l'énergie libre de la réaction est $\Delta F = -RT\, ln\, K$, où $R = 1{\cdot}9885$ calories/degré; on obtient ainsi: $\Delta F = +\,19{\cdot}500$ calories environ à 45° C.

La mesure du potentiel a été effectuée dans une solution dont on connaît non pas les activités en ions succinate et fumarate, mais les concentrations totales en ions et molécules de chacun des acides. Toutefois les erreurs introduites en utilisant dans le calcul de la constante d'équilibre les concentrations totales en ions et molécules au lieu des activités en ions succinate⁼ et fumarate⁼ se compensent suffisamment.

Les mesures les plus récentes de l'énergie libre de l'oxydation de l'acide succinique en acide fumarique sont dues à Borsook et Schott, et donnent comme valeur à 25° C.: $\Delta F = +20{\cdot}140$ calories.

Cette méthode, jusqu'en ces derniers temps, n'avait été appliquée que dans le cas de l'équilibre acide succinique $\rightleftharpoons$ acide fumarique par Quastel et Whetham (1924), d'une part, Thunberg (1925), d'autre part, et, avec une précision croissante, par Lehmann (1930) et enfin Borsook et Schott (1931). Cette méthode cependant doit pouvoir être utilisée pour toutes les oxydoréductions que l'on sait réaliser à l'aide de déshydrases.

La théorie des catalyseurs indique que, dans chaque cas, la réaction régressive doit, au voisinage de l'équilibre, être accélérée aussi bien que la réaction progressive. La méthode qui a permis de mettre en évidence l'équilibre dans le cas de l'acide succinique est générale; mais nous allons voir que les conditions de son emploi sont assez étroites, et pour cela il faut tout d'abord préciser ce que l'on entend par potentiel normal d'oxydoréduction d'un système et par indicateur d'oxydoréduction.

3. *Potentiel normal d'oxydoréduction.*

Considérons l'équilibre dont il a déjà été question entre les ions bleu⁺ et leuco⁻ du bleu de méthylène:

$$\text{bleu}^+ + 2e \rightleftharpoons \text{leuco}^-.$$

En substituant dans l'équation (9) l'activité électronique du système $\text{bleu}^+ \rightleftharpoons \text{leuco}^-$, on obtient la relation:

$$E_h = -\frac{RT}{2\mathfrak{F}}\, ln\, K - \frac{RT}{2\mathfrak{F}}\, ln \frac{[\text{leuco}^-]}{[\text{bleu}^+]} \qquad \text{......(11).}$$

E_h est le potentiel d'oxydoréduction du système $\text{bleu}^+ \rightleftharpoons \text{leuco}^-$ par rapport à l'électrode normale d'hydrogène. Le premier terme du second membre n'est pas autre chose que le produit par $\frac{RT}{2\mathfrak{F}}$ du logarithme de la constante d'équilibre de la réaction, et nous pouvons écrire

$$E_h = E_0 - \frac{RT}{2\mathfrak{F}}\, ln \frac{[\text{leuco}^-]}{[\text{bleu}^+]} \qquad \text{......(12).}$$

Une telle équation qui représente le potentiel du mélange d'ions bleu⁺ et leuco⁻, en fonction de leur pourcentage respectif, est une courbe en **S** (fig. 1). Quand la forme oxydée bleu⁺ et la forme réduite leuco⁻ ont des activités égales, on a:

$$[\text{bleu}^+] = [\text{leuco}^-]$$
$$E_h = E_0.$$

E_0 est ce que l'on appelle le potentiel normal du système $\text{bleu}^+ \rightleftharpoons \text{leuco}^-$.

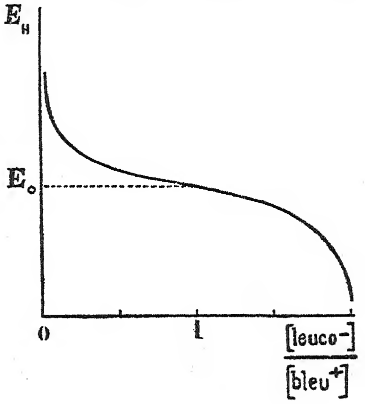


Fig. 1.

Mais les activités [bleu⁺] et [leuco⁻] d'un mélange donné ne sont généralement pas connues. Ce que nous connaissons, c'est la quantité de bleu de méthylène, et celle de son leucodérivé que nous pouvons mettre en solution. Ces molécules se dissocient plus ou moins suivant la teneur de la solution en d'autres ions, spécialement en ions H^+. Si l'on veut tracer une courbe du potentiel en fonction de la composition du mélange de bleu de méthylène et de son leucodérivé, il faut chercher comment ce potentiel varie en fonction du rapport de la totalité (S_0) des molécules et ions de la forme oxydée et de la totalité (S_r) des molécules et ions de la forme réduite. Puisque le potentiel du système est déterminé par le rapport des activités des ions bleu$^+$ et leuco$^-$, il faut rechercher comment l'activité [bleu$^+$] dépend de (S_0) et l'activité [leuco$^-$] de (S_r).

Nous avons vu que les ions bleu$^+$ proviennent de la dissociation de la forme oxydée du bleu de méthylène qui est une base. On a donc un équilibre que l'on peut représenter par le schéma suivant, en désignant par le mot bleu la molécule de bleu de méthylène non dissociée:

$$\text{bleu} \rightleftharpoons \text{bleu}^+ + \text{OH}^-.$$

Soit K_0 la constante de cet équilibre:

$$K_0 = \frac{[\text{bleu}^+]}{[\text{bleu}]}\,[\text{OH}^-].$$

Il y a intérêt à exprimer cette relation en fonction non pas des ions OH^- mais des ions H^+. Ces deux sortes d'ions sont en équilibre avec l'eau. Si l'on appelle K_w la constante de dissociation de l'eau, on obtient:

$$[\text{bleu}] = \frac{[\text{bleu}^+]\,K_w}{K_0\,[\text{H}^+]},$$

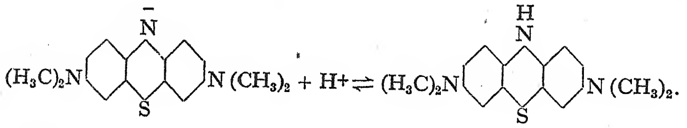
et en admettant que les activités diffèrent peu des concentrations:

$$(S_0) = [\text{bleu}] + [\text{bleu}^+] = [\text{bleu}^+]\,\frac{K_0\,[\text{H}^+] + K_w}{K_0\,[\text{H}^+]} \qquad \text{......(13).}$$

Quant à la forme réduite du bleu de méthylène, elle comprend les ions leuco$^-$ que nous avons considérés. Mais ces anions sont en très petite proportion. Ils fixent des ions H^+ et donnent la leucobase du bleu de méthylène, suivant un équilibre dont nous appellerons K_1 la constante:

$$K_1 = \frac{[\text{leuco}^-]\,[\text{H}^+]}{[\text{leuco}]};$$

[leuco] représente l'activité des molécules non dissociées de la leucobase du bleu de méthylène. Le schéma ci-dessous montre en quoi consiste cette dissociation:



Les molécules de leucobase renferment encore des groupements aminés qui jouissent de propriétés basiques faibles. Elles donnent donc de nouveaux ions, dont il faut tenir compte. Remarquons, avec Bronstedt, qu'une base faible, c'est à dire qui émet en solution peu d'ions OH^- peut être considérée tout aussi bien comme un acide fort. Ainsi la base faible $NH_2C_6H_5$ se comporte comme si elle était le produit de dissociation de l'acide fort $^+NH_3C_6H_5$:

$$^+NH_3C_6H_5 \rightleftharpoons NH_2C_6H_5 + H^+.$$

En considérant de cette manière les groupements aminés du bleu de méthylène réduit on écrit les équilibres:

$$K_2 = \frac{[\text{leuco}]\,[H^+]}{[\text{leuco}^+]} \qquad K_3 = \frac{[\text{leuco}^+]\,[H^+]}{[\text{leuco}^{++}]},$$

ce qui a l'avantage de ne pas faire intervenir explicitement les ions OH^- et de permettre la combinaison de toutes ces équations. On a en effet:

$$(S_r) = [\text{leuco}^-] + [\text{leuco}] + [\text{leuco}^+] + [\text{leuco}^{++}] \qquad \text{......(14).}$$

En substituant dans (12) les valeurs de [bleu$^+$] et de [leuco$^-$] tirées de (13) et de (14) après avoir mis dans cette dernière équation le terme [leuco$^-$] en facteur, on obtient:

$$E_h = E_0 - \frac{RT}{2\mathfrak{F}} \, ln \, \frac{K_1K_2K_3}{K_0} - \frac{RT}{2\mathfrak{F}} \, ln \, \underbrace{\frac{K_0\,[H^+] + K_w}{K_1K_2K_3\,[H^+] + K_2K_3\,[H^+]^2 + K_3\,[H^+]^3 + [H^+]^4}}_{A} - \frac{RT}{2\mathfrak{F}} \, ln \, \frac{(S_r)}{(S_0)}.$$

A un pH donné, cette équation se ramène à la suivante:

$$E_h = E_0' - \frac{RT}{2\mathfrak{F}} \, ln \, \frac{(S_r)}{(S_0)} \qquad \text{......(15),}$$

qui est du même type que l'équation (12), mais dont la constante E_0', que l'on peut encore appeler potentiel normal du système bleu de méthylène $\rightleftharpoons$ leucobase, et qui correspond à l'égalité des concentrations en forme oxydée et en forme réduite, n'a plus la même signification thermodynamique simple que le potentiel normal E_0 du système bleu$^+$ $\rightleftharpoons$ leuco$^-$, et varie avec le pH.

Cette variation est illustrée par le graphique (fig. 2) qui représente les valeurs de E_0' en fonction du pH. Remarquons en passant que ce graphique permet, comme l'ont fait M. Clark, Cohen et Gibbs (1925), de déterminer les diverses constantes de dissociation. Sans entrer dans les détails, on voit que, en milieu alcalin, et jusqu'à pH 5·8 environ, E_0' augmente de + 0·030 volt à 30° C., c'est à dire de $\frac{1}{0{\cdot}4343} \frac{RT}{2\mathfrak{F}} \log 10$ quand le pH diminue d'une unité, c'est donc que le terme A a été divisé par 10.

Or le numérateur du terme A, soit $K_0\,[H^+]$, K_w étant négligeable vis-à-vis de $K_0\,[H^+]$ puisqu'on a affaire à une base forte, est multiplié par 10 à chaque variation d'une unité de pH; si donc A a diminué 10 fois, c'est que le numérateur de A est devenu 100 fois plus grand, c'est à dire a augmenté proportionnellement à $[H^+]^2$. C'est dire encore que tous les termes en $[H^+]$, $[H^+]^3$, $[H^+]^4$, sont négligeables vis-à-vis de celui en $[H^+]^2$. Le terme en $[H^+]$ est nécessairement très petit puisqu'il contient le facteur K_1, et que cette constante correspond à la dissociation en ions leuco⁻ qui, nous l'avons vu, n'existent qu'en très petite quantité. Pour que $K_3 [H^+]^3$ soit plus petit que $K_2K_3 [H^+]^2$, il faut que K_2 soit plus grand que $[H^+]$; d'autre part, K_3 est plus grand que K_2 car K_3 se rapporte à la deuxième dissociation basique donc à un acide plus fort que celui qui correspond à la première dissociation basique. Ainsi la pente de la courbe E_0' : *p*H sera de 0·030 volt par unité de *p*H tant que $[H^+]$ ne sera pas égal à K_2. A ce moment, à *p*H 5·85, il y aura un point anguleux. On trouve un autre point anguleux à *p*H 4·52 qui correspond au moment où la valeur de l'activité en ions H^+ atteint la valeur de K_3.

Enfin, en milieu acide, à partir de *p*H 4·5, E_0' augmente de + 0·090 volt par unité de *p*H. C'est dire que, dans cette région, c'est le terme en $[H^4]$ qui domine au dénominateur de A; ce qui est évident puisque les constantes de dissociation K_2 et K_3 sont chacune inférieure à $10^{-4{\cdot}5}$.

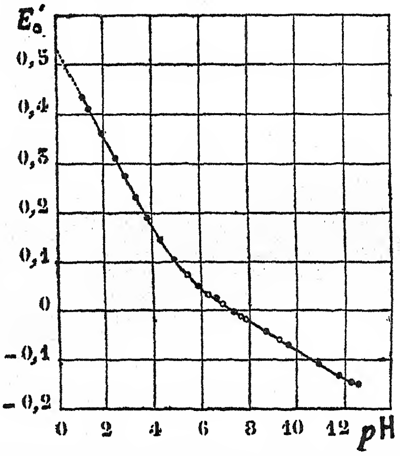


Fig. 2. Variation de la constante E_0' en fonction du *p*H (d'après Clark, Cohen et Gibbs).

4. *Indicateurs d'oxydoréduction.*

Les différentes courbes obtenues à divers *p*H forment donc une famille de courbes en **S** dont les points d'inflexion E_0' varient avec le *p*H d'une manière qui dépend de la dissociation, en tant qu'acides ou bases faibles, des formes réduite ou oxydée. Ces courbes peuvent être obtenues très simplement soit en mesurant le potentiel de mélanges en proportions variables des formes oxydée et réduite, soit en faisant un titrage électrométrique: on ajoute progressivement un agent oxydant au système d'oxydoréduction réduit, ou un agent réducteur au système oxydé, et l'on porte les potentiels du système en fonction des quantités de réactif ajoutées. Il faut seulement que l'agent de titrage soit *fort* par rapport au système titré, c'est à dire que les potentiels normaux de cet agent et du système soient très différents.

Si les corps en équilibre d'oxydoréduction ont une couleur différente suivant qu'ils sont oxydés ou réduits, leur variation de couleur peut servir à déterminer le potentiel d'oxydoréduction d'un milieu, pourvu que ces corps soient employés à une concentration assez faible pour ne pas déplacer l'équilibre du milieu. C'est là le principe de la détermination des potentiels d'oxydoréduction par l'emploi des indicateurs. On doit à M. Clark et à ses collaborateurs la création de cette méthode. Les travaux originaux de M. Clark et de ses collaborateurs ont été publiés dans les Public Health Reports depuis 1923. Toutes les données sur les indicateurs d'oxydoréduction connus jusqu'en 1930 ont été réunies dans la monographie de R. Wurmser (1930). Depuis cette date ont encore paru de nouvelles déterminations de Michaelis et Eagle (1930), Michaelis (1931), Cohen et Preisler (1931), Friedheim et Michaelis (1931), Letort (1932).

5. *Propriétés d'un système oxydoréducteur au voisinage de son potentiel normal.*

Avant d'utiliser l'échelle d'indicateurs d'oxydoréduction à la détermination des énergies libres des réactions, il faut encore mentionner une propriété importante que possèdent les systèmes oxydoréducteurs au voisinage de leur potentiel normal.

Prenons encore une fois comme exemple le système formé par le bleu de méthylène. Son potentiel, en fonction de la composition, est, à un pH donné:

$$E_h = E_0' - \frac{RT}{2\mathfrak{F}} \, ln \frac{(S_r)}{(S_0)} = E_0' - \frac{RT}{2\mathfrak{F}} \, ln \frac{(S - S_0)}{(S_0)},$$

en appelant (S) la quantité totale de bleu de méthylène réduit et oxydé. La variation $\frac{dE}{d(S_0)}$ du potentiel quand la quantité de la forme oxydée augmente par rapport à la forme réduite est minima, comme cela apparaît sur la courbe (fig. 1), quand $(S_0) = \frac{1}{2}(S)$, c'est à dire au potentiel normal du système[1]. En ce point le système est tamponné au point de vue oxydoréduction, il est, selon l'expression de Clark "poised."

Il est important de noter qu'une électrode inerte plongée dans une solution d'un système oxydoréducteur indique toujours un potentiel relativement voisin du potentiel normal de ce système, parce qu'il faudrait, pour s'en écarter beaucoup, une pureté extrême dans l'une des formes réduite ou oxydée du système.

6. *Emploi généralisé des indicateurs comme corps électroactifs.*

Nous avons vu que, pour déterminer l'énergie libre d'un système constitué par un corps AH_2 non actif sur une électrode inerte et par son produit d'oxydation A, il suffit, en principe, de le faire réagir, au moyen d'un catalyseur convenable, avec un autre corps X *électroactif*, c'est à dire qui soit capable d'échanger des électrons avec un métal inerte et qui soit lui-même en équilibre avec son produit de réduction X′. Nous avons envisagé le cas où AH_2 est l'acide succinique, A l'acide fumarique, X le bleu de méthylène, et nous avons dit que, pour généraliser la méthode à d'autres oxydoréductions, il fallait réaliser une condition que nous sommes maintenant en mesure de préciser. Il s'agit en effet de mesurer le potentiel du système intermédiaire $X \rightleftharpoons X'$, une fois l'équilibre atteint. On peut y parvenir de deux manières:

---

[1] La pente en ce point a pour valeur $2\frac{RT}{\mathfrak{F}}$ soit, à 30° C., 0·052 volt; si, au lieu de deux équivalents, la réaction portait sur n équivalents, la pente serait $4\frac{RT}{n\mathfrak{F}}$.

1°. *Colorimétriquement*, si X est un indicateur d'oxydoréduction. Par exemple, dans l'équilibre acide succinique ⇌ acide fumarique, cité plus haut, on trouve que, si le rapport du succinate au fumarate est 1, celui du bleu de méthylène réduit au bleu de méthylène oxydé est 3. D'après l'équation (15), en prenant la valeur de E_0' à 45° C. et *p*H 7·2, le potentiel est − 0·030 volt. Mais il est clair que la couleur de l'indicateur ne peut servir à mesurer le potentiel que si l'indicateur n'est ni totalement oxydé, ni totalement réduit, c'est à dire si l'équilibre s'établit au voisinage de son potentiel normal.

2°. *Électrométriquement*, en mesurant le potentiel d'une électrode inerte plongée dans le mélange. Mais, pour des raisons d'ordre cinétique, une électrode inerte plongée dans un système oxydoréducteur très éloigné de son potentiel normal ne peut donner des indications exactes sur l'état de ce système que si ce dernier existe en masse très importante, ce qui doit être évité pour diverses raisons, en particulier pour ne pas inhiber le catalyseur quand celui-ci est une diastase. En outre le système intermédiaire n'atteint pratiquement son équilibre avec le système $AH_2 \rightleftharpoons A$ que si cet équilibre ne correspond pas à un rapport extrêmement faible de la forme oxydée X à la forme réduite X', sinon la concentration de X est si petite que la vitesse de réduction devient pratiquement nulle.

La détermination d'un équilibre tel que $AH_2 \rightleftharpoons A$ suppose donc la recherche préliminaire d'un indicateur d'oxydoréduction approprié. Wurmser et De Boe (1932) ont entrepris cette recherche dans le cas de l'équilibre supposé entre l'acide lactique et l'acide pyruvique:

$$CH_3.CHOH.COO^- \rightleftharpoons CH_3.CO.COO^- + H_2.$$

Le catalyseur choisi était la préparation diastasique de Stephenson (1928), qui a donné d'excellents résultats. Cette préparation consiste en un autolysat de *B. coli*. En présence de toluène, elle ne catalyse pas l'oxydation de l'acide pyruvique tandis qu'elle entraîne une oxydation rapide de l'acide lactique. Quand on soumet à l'action de cet autolysat, en présence de lactate de sodium, à *p*H 7·4, une série d'indicateurs d'oxydoréduction dont les potentiels normaux, à ce *p*H, sont connus, le bleu de méthylène, les sulfonates d'indigo, le bleu de Nil et le violet de crésyle sont réduits. La phénosafranine, le vert Janus et le rouge neutre restent colorés.

Comme tous ces indicateurs, à l'exception des sulfonates d'indigo, sont de constitution chimique très analogue, il est logique de penser que la non-réduction des colorants de potentiel normal plus négatif que celui du violet de crésyle est due, non pas à une propriété sélective de la diastase, mais au fait que l'équilibre entre l'acide lactique et l'acide pyruvique correspond à un potentiel normal compris entre celui du violet de crésyle et celui de la phénosafranine, soit entre − 0·180 et − 0·260 volt.

D'autre part on peut suivre électrométriquement la variation du potentiel d'une électrode inerte dans un mélange d'autolysat, de lactate, et de divers indicateurs d'oxydoréduction en milieu fortement tamponné à *p*H 7·4 à 37°. Ces mesures ont été effectuées dans un tube de Thunberg, c'est à dire dans un tube à essai muni d'une tubulure latérale qui permet de faire le vide. Le tube portait, en outre, une électrode de platine montée dans un rodage, et un tube capillaire contenant de la gélose saturée de chlorure de potassium. Ce capillaire formait le pont réunissant la solution avec une électrode de calomel. On mesurait, en fonction du temps, les potentiels d'une électrode de platine plongée dans la solution. Les courbes ainsi obtenues donnent le potentiel du système oxydoréducteur contenu dans la solution et indiquent par conséquent la valeur du rapport de la forme réduite à la forme oxydée de l'indicateur puisque les acides lactique et pyruvique sont par eux-mêmes inactifs sur l'électrode. On constate que le potentiel devient rapidement négatif et s'abaisse en 3 ou 4 heures jusqu'au voisinage de – 0·120 volt, avec les colorants tels que la phénosafranine, le violet de crésyle et le bleu de Nil. Avec le bleu de méthylène, la courbe présente un plateau correspondant au potentiel normal de ce colorant. A partir de – 0·120 volt, la courbe s'abaisse lentement et ne se stabilise qu'en présence de toluène, à – 0·170 volt dans le cas de la phénosafranine, et à – 0·200 volt avec le violet de crésyle et le bleu de Nil (fig. 3; courbe I).

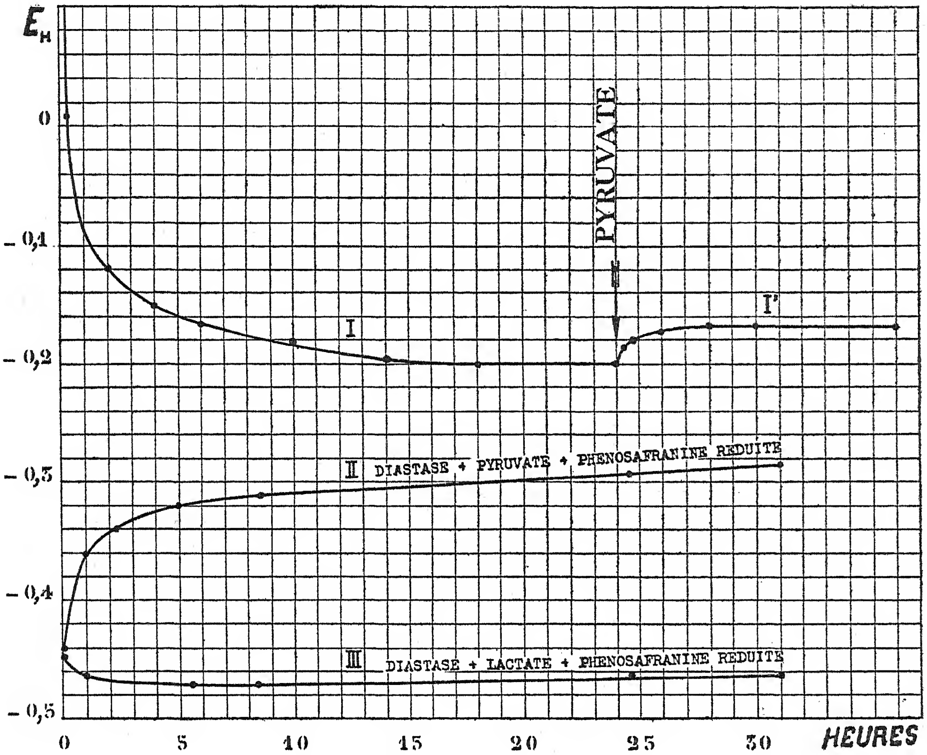


Fig. 3. Équilibre entre les acides lactique et pyruvique (d'après Wurmser et N. Mayer).

Tandis que le potentiel mesuré en présence de bleu de Nil et de violet de crésyle correspond bien à l'état de réduction de ces colorants dans le mélange final, le potentiel plus positif mesuré en présence de phénosafranine indique que le système acide lactique ⇌ acide pyruvique est en dehors du domaine de potentiel où des quantités notables de phénosafranine réduite commenceraient à apparaître. La valeur probable du potentiel normal du système acide lactique ⇌ acide pyruvique est donc d'environ $-0{\cdot}200$ volt à *p*H 7·4, et à 37° C.

Pour s'assurer que le domaine de potentiel compris entre $-0{\cdot}170$ et $-0{\cdot}260$ volt est bien un domaine d'équilibre pour le système acide lactique ⇌ acide pyruvique, il fallait montrer que, tandis que l'acide lactique réduit le violet de crésyle dont le potentiel normal est de $-0{\cdot}187$ volt, l'acide pyruvique oxyde le leucodérivé de la phénosafranine dont le potentiel normal est de $-0{\cdot}265$ volt, au *p*H et à la température de l'expérience.

L'expérience a été réalisée de la manière suivante (Wurmser et N. Mayer, 1932). On a utilisé comme catalyseur, ainsi que dans les recherches antérieures, la diastase de Stephenson. 1 c.c. de l'autolysat de *B. coli* qui constitue ce catalyseur est dilué avec 1 c.c. d'un tampon de phosphate à *p*H 7·4, saturé de toluène. On ajoute 1 c.c. de pyruvate de sodium 1/20 *M*, et enfin 1 c.c. d'une solution de phénosafranine 10^{-3} *M* réduite avec un léger excès d'hydrosulfite de sodium. On peut facilement effectuer l'expérience dans un tube renversé sur une cuve de mercure. A 37° C., la phénosafranine se recolore en des temps variant de 15 minutes à deux heures suivant l'activité de la diastase et l'excès d'hydrosulfite introduit qui a pour but de fixer l'oxygène dissous dans la solution de pyruvate et la préparation diastasique. La recoloration se produit beaucoup plus lentement en l'absence de diastase ou en présence de diastase bouillie. Enfin des tubes témoins contenant, au lieu de pyruvate, une solution équivalente de lactate, restent toujours incolores.

Le graphique (fig. 3) représente la marche de la réaction dans le sens progressif acide lactique → acide pyruvique, en présence de violet de crésyle comme accepteur (courbe I), et dans le sens régressif, acide pyruvique → acide lactique, en présence de phénosafranine réduite comme donateur d'hydrogène (courbe II). Le potentiel normal de ce dernier colorant étant beaucoup plus négatif que celui du système acide lactique ⇌ acide pyruvique, la courbe II ne peut pas servir à déterminer cet équilibre. Par contre, en ajoutant à la solution de lactate qui a atteint son équilibre avec le violet de crésyle (courbe I) une quantité de pyruvate telle que le rapport [pyruvate]/[lactate] devienne égal à 7, on obtient (courbe I′) un nouvel équilibre dont le potentiel correspond à $-0{\cdot}165$ volt. L'activité de l'hydrogène s'obtient en portant dans la relation (10) le potentiel $-0{\cdot}165$ volt et le *p*H corrigé pour 37° C. On obtient $rH_2 = 9{\cdot}34$, c'est à dire $[H_2] = 10^{-9{\cdot}34}$. En portant cette valeur dans la constante d'équilibre K de la réaction, on obtient:

$$K = \frac{[CH_3.CO.COO^-]}{[CH_3.CHOH.COO^-]}[H_2] = 7{\cdot}10^{-9{\cdot}34} = 10^{-8{\cdot}49}.$$

L'énergie libre de la réaction, quand tous les constituants sont à l'activité 1, les corrections de dissociation étant négligeables, a pour valeur:

$$\Delta F = -RT\,ln\,K = +1{\cdot}99 \times 310 \times 2{\cdot}3 \times 8{\cdot}49 = +12040 \text{ calories.}$$

La détermination des potentiels d'oxydoréduction étendue aux corps non-actifs sur l'électrode grâce à l'emploi de systèmes intermédiaires actifs, de potentiel normal approprié, permettra de connaître les énergies libres de nombreuses autres réactions d'oxydation que l'on sait catalyser par des déshydrases et dont les données énergétiques manquaient jusqu'ici. Cette détermination peut, d'ailleurs, être très difficile. Ainsi dans l'oxydation de l'aldéhyde acétique en acide acétique, en présence de xanthinoxydase, tous les indicateurs d'oxydoréduction actuellement bien étudiés, c'est à dire jusqu'au rouge neutre, sont réduits, et l'échelle des indicateurs devra être étendue pour permettre la mesure de l'énergie libre de cette réaction.

II. LES ÉQUILIBRES D'OXYDORÉDUCTION DANS LES MILIEUX CELLULAIRES.

7. *Signification du potentiel d'oxydoréduction intracellulaire.*

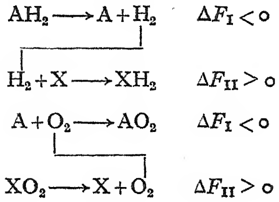
Si l'étude des potentiels d'oxydoréduction ne devait servir qu'à ce genre de détermination, elle aurait déjà un rôle important à jouer en biochimie. Mais les données thermodynamiques ne nous renseignent que sur les possibilités de réaction; ce sont les facteurs qui influent sur la vitesse d'évolution des systèmes qui, en général, dominent le déterminisme des réactions, et ce sont eux les plus importants à connaître. Il n'en est plus de même si les systèmes sont en équilibre; dans ce cas, les facteurs thermodynamiques déterminent entièrement leur état.

Nous avons vu que les acides succinique et fumarique, lactique et pyruvique, sont en équilibre en présence de certaines diastases avec des accepteurs et donateurs d'hydrogène tels que les indicateurs d'oxydoréduction. Il n'est pas hasardeux d'imaginer que, dans les cellules, où ces indicateurs n'existent pas, il se trouve par contre des substances qui peuvent, en présence des mêmes diastases, recevoir et donner de l'hydrogène à ces acides. Ainsi s'établira entre eux un équilibre qui contribuera à ce que l'on peut appeler le maintien de la forme chimique des organismes. Si donc il existe dans les organismes des substances en équilibre d'oxydoréduction jouant un rôle physiologique, la détermination des potentiels d'oxydoréduction des milieux cellulaires prendra une signification toute nouvelle.

J'ai été conduit, il y a près d'une dizaine d'années, à cette hypothèse en cherchant à rendre compte de la permanence des réactions de synthèse dans les cellules. Comme une sorte de fond sur lequel se détachent les réactions vives telles que la respiration et la fermentation glycolytique, se poursuivent des réactions lentes mais dont l'importance est essentielle qualitativement, puisque leurs produits sont les constituants de la matière vivante. La permanence de ces réactions doit être assurée par la cellule au même titre que sa disposition morphologique.

Ces réactions sont d'une manière générale des réductions correspondant à un accroissement d'énergie libre. La compensation nécessaire est réalisée par couplage avec des oxydations.

Sans chercher à préciser jusqu'à nouvel ordre le mécanisme de ces couplages, on peut dire que ceux-ci ne doivent être possibles que si l'oxydation complète du réducteur aux dépens de l'oxygène moléculaire est évitée, autrement dit si l'on a toujours plus ou moins un couplage de réactions du type:



$$\begin{array}{ll} AH_2 \longrightarrow A + H_2 & \Delta F_{I} < 0 \\ H_2 + X \longrightarrow XH_2 & \Delta F_{II} > 0 \\ A + O_2 \longrightarrow AO_2 & \Delta F_{I} < 0 \\ XO_2 \longrightarrow X + O_2 & \Delta F_{II} > 0 \end{array}$$

en représentant par ΔF_{I}, ΔF_{II} les variations d'énergie libre.

Si tel est le mécanisme général des synthèses, le maintien de la vie, au moins en ce qui concerne la réparation de sa substance, apparaît comme le résultat d'une compétition entre les oxydations qui se font aux dépens de l'oxygène libre et celles qui se font aux dépens d'autres sortes de molécules. Il semble donc que la proportion entre ces deux types d'oxydation doit être assurée de quelque manière (Wurmser, 1923).

L'orientation des molécules vers l'un ou l'autre de ces destins est liée à l'existence des diastases. Mais l'existence de catalyseurs spécifiques, même localisés en certains domaines des cellules, permet de concevoir l'évolution simultanée des divers types d'oxydation plus aisément que le maintien de leurs vitesses relatives. C'est pourquoi on pouvait se demander s'il n'existait pas une relation entre la possibilité pour certaines synthèses de s'effectuer au sein des cellules vivantes et une propriété générale des milieux cellulaires qui serait d'avoir toujours un potentiel d'oxydoréduction maintenu entre des limites définies.

Il convient de définir ce que nous entendons par potentiel d'oxydoréduction des milieux cellulaires. Il existe dans la cellule de nombreux corps qui, en présence de catalyseurs convenables, peuvent mobiliser leur hydrogène. Comme nous l'avons vu dans la première partie de cet exposé, ces corps peuvent être actifs ou inactifs vis-à-vis d'une électrode. Mais dans les deux cas on peut définir un potentiel d'oxydoréduction.

Thermodynamiquement, on peut parler par exemple du potentiel d'oxydoréduction du système acide succinique ⇄ acide fumarique bien qu'un mélange de ces deux corps ne soit pas actif sur une électrode et ne le devienne qu'en présence d'une diastase spécifique convenable. Considérons par exemple dans le milieu cellulaire les acides succinique et fumarique, lactique et pyruvique, les potentiels d'oxydoréduction de ces deux systèmes seront égaux entre eux dans la mesure où il existera au sein des cellules un constituant commun de ces réactions, jouant, au niveau des diastases respectives, le rôle d'accepteur et de donateur. En l'absence de tels intermédiaires, il pourra y avoir dans les cellules des groupes de systèmes en équilibre, mais à des niveaux différents. Dans ces conditions, la notion de potentiel d'oxydoréduction d'un milieu cellulaire n'a de sens que si l'on précise la nature des systèmes concernés. Les seuls systèmes dont nous pourrons déterminer directement le potentiel sont ceux qui sont effectivement, dans les conditions physiologiques, en équilibre électrochimique, c'est à dire dont les constituants ont leur activité réglée par l'activité électronique du milieu. Les oxydations et les réductions des corps du métabolisme apparaissent dans les bilans chimiques comme des transports d'hydrogène, et il semble bien depuis les travaux de Wieland (1922) que les actions catalytiques qui rendent possibles ces réactions consistent en une mobilisation de cet hydrogène. D'après la définition du potentiel d'oxydoréduction cellulaire qui vient d'être donnée on peut donc dire que les oxydations et réductions qui, suivant leur masse, déterminent ce potentiel ou en dépendent sont celles où intervient de l'hydrogène électroactif, c'est à dire en équilibre avec ses ions suivant la réaction (II). Au point de vue des relations entre le potentiel d'oxydoréduction et les réactions chimiques qui s'effectuent dans les cellules, il y a donc intérêt à expliciter l'activité de l'hydrogène électroactif. C'est pourquoi on emploiera avec avantage pour exprimer le niveau dans l'échelle d'oxydoréduction la notation de M. Clark, définie plus haut et qui consiste à representer par $r\mathrm{H}_2$ le cologarithme de cette activité.

Dans la suite nous serons conduit à rechercher si les constituants cellulaires, dont nous avons envisagé la synthèse, sont en équilibre avec des systèmes électroactifs, ce qui implique, au point de vue du mécanisme chimique, des conséquences qu'il serait trop long de développer ici, et qui donnent aux réactions entre ions une grande importance dans les réactions biologiques.

Nous allons maintenant rencontrer dans la mesure même du potentiel ainsi défini certaines difficultés.

Pratiquement, on ne peut guère employer pour les études intracellulaires la technique électrométrique. On utilise la méthode de Needham et Needham (1925, 1926) qui consiste à microinjecter des indicateurs, ou bien, suivant la manière primitive d'Ehrlich, on utilise ceux de ces indicateurs qui, étant des colorants vitaux, pénètrent d'eux-mêmes dans les cellules et s'y fixent.

Il faut que la mesure ne perturbe pas le potentiel du système cellulaire. Or, nous avons vu que l'addition de colorants à des donateurs d'hydrogène en présence de diastases convenables peut rendre électrochimiquement actif un système oxydoréducteur qui ne l'était pas sans cette addition. En outre, les colorants peuvent créer de nouvelles liaisons entre l'oxygène moléculaire et les systèmes oxydoréducteurs. En ce qui concerne les actions diastasiques, Rapkine et Wurmser (1927) ont montré qu'elles ne semblent pas intervenir, car l'addition de donateurs comme le succinate, le pyruvate de sodium, ou le glucose, au colorant injecté, ne modifie en rien la vitesse de réduction. Quant à la liaison avec O_2, on peut tenir compte du fait que la microinjection donne les mêmes résultats, que la mesure soit faite dans l'oxygène ou dans l'azote, pourvu qu'un séjour prolongé dans ce gaz inerte ne modifie pas l'état du milieu cellulaire lui-même.

Les difficultés dont il a été question jusqu'ici ne seraient donc pas très redoutables si les mesures étaient toujours rapides. Mais, à mesure que l'indicateur employé a un potentiel qui se rapproche de celui du système mesuré, les vitesses de décoloration diminuent. En même temps, le système devient de moins en moins capable de réduire des quantités importantes de colorant.

Considérons en effet une courbe de titrage électrométrique d'un système oxydoréducteur (fig. 4). Nous avons vu que cette courbe a une forme en **S** et que son palier correspond au potentiel normal du système. Si le potentiel de ce système, que nous supposerons être celui du milieu cellulaire, correspond au point A, les colorants dont le potentiel normal est compris entre A et B seront réduits mais en très petite quantité. Au contraire, une quantité équivalente à la moitié du système réducteur pourra être réduite si le colorant a son potentiel normal égal à E_0'. Enfin, les colorants dont le potentiel normal est plus positif que E_0' pourront être réduits en proportion de plus en plus grande ; finalement, les colorants de potentiel normal plus positif que C seront réduits en quantité pratiquement équivalente à celle du système réducteur.

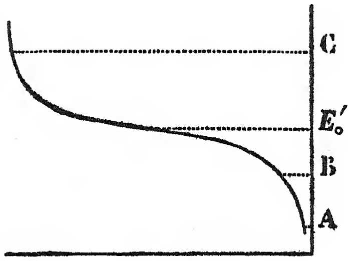


Fig. 4.

Le point A correspond au potentiel limite du système et c'est le chiffre qui serait donné par une détermination électrométrique, l'électrode se comportant à ce point de vue comme un accepteur de très petite masse. Mais on conçoit que des quantités notables de colorant sont nécessaires pour que la couleur apparaisse dans une cellule microinjectée, d'épaisseur minime, ou même lorsque l'on opère par coloration vitale. La mesure colorimétrique indiquera donc toujours un potentiel limite trop positif. Et des différences observées entre deux milieux peuvent correspondre à des différences non dans les potentiels, mais dans les masses des systèmes réversibles. C'est ainsi que les différences de potentiel limite observées dans les divers tissus (Aubel et Wurmser, 1929) peuvent être interprétées de ces deux manières.

Toutefois, si l'on pense que l'intérêt des mesures de potentiel est de nous faire connaître les possibilités de travail chimique qui caractérisent le milieu étudié, ce qui importe, ce n'est pas tant de connaître avec précision le niveau du potentiel limite ; il faut surtout connaître la position des potentiels normaux des systèmes oxydoréducteurs existant dans les cellules ainsi que la masse de ces systèmes.

La détermination la plus urgente est donc le titrage électrométrique ou colorimétrique de ces systèmes, qui peut seul nous donner la position des potentiels caractéristiques. Le premier système oxydoréducteur qui ait été étudié, parmi ceux qui sont susceptibles d'intervenir dans les milieux cellulaires, est le système constitué par le glutathion et son produit d'oxydation. Cependant nous commencerons par l'étude d'un autre type de substances, dérivées des glucides, parce que les potentiels d'oxydoréduction que l'on mesure dans les solutions de ces substances sont, malgré les apparences, plus simples à interpréter.

8. *Potentiel des solutions de glucides.*

Le potentiel limite. Quand on plonge une électrode inerte dans une solution d'un glucide réducteur maintenue à l'abri de l'oxygène, on observe que son potentiel devient de plus en plus négatif et atteint une valeur limite au bout d'un temps d'évolution qui diminue avec l'alcalinité et la température et qui varie de quelques minutes à quelques mois. La mesure de la différence de potentiel qui s'établit entre une électrode inerte et des solutions de glucides a été effectuée par Goard et Rideal en 1924 dans les solutions alcalines. Ces auteurs observaient un potentiel passant au cours du temps par un minimum, le potentiel était différent pour chaque glucide; mais en réalité (Wurmser et Geloso (1928, 1931), Nelicia Mayer (1929)), si l'on tient compte des modifications de concentration en ions H^+ qui se produisent dans les solutions, tous les glucides ont, dans la limite de précision des expériences, le même potentiel limite. Ce potentiel limite obéit aux lois suivantes:

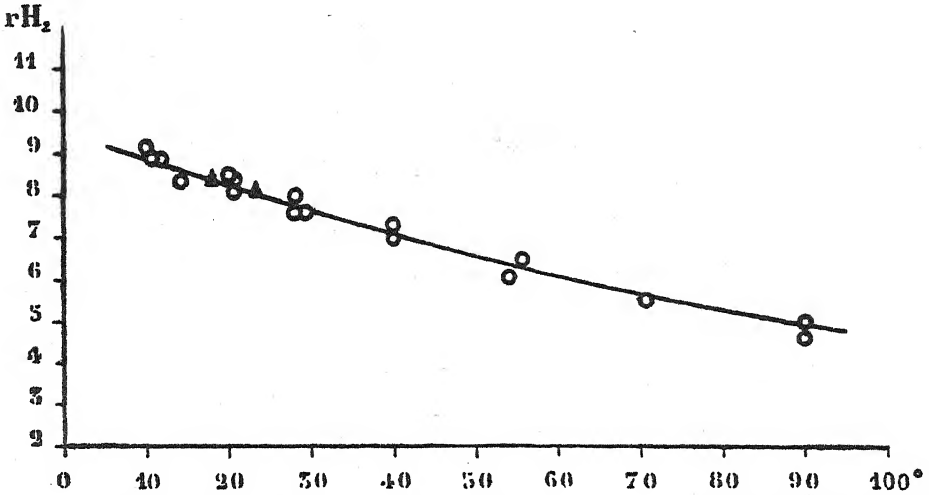


Fig. 5. Valeurs de rH_2 entre 10° et 90° C., permettant de calculer le potentiel limite d'une solution de glucide réducteur, à tout pH compris entre 7 et 11 (d'après Wurmser et Geloso).

(*a*) *Influence de la réaction.* Si l'on porte en abcisses les valeurs du pH final des solutions, et en ordonnées les potentiels limites de l'électrode, on obtient des courbes sensiblement linéaires qui peuvent être représentées entre pH 7 et pH 11, par l'équation:

$$E_h = -\ 0{\cdot}00020\,T\,p\mathrm{H} - 0{\cdot}00088\,T + 0{\cdot}50 \pm 0{\cdot}02 \text{ volt.}$$

En calculant la valeur de rH_2 correspondant à ce potentiel, d'après la relation (10), on trouve que cette valeur est indépendante du pH et varie avec la température suivant l'expression:

$$rH_2 = \frac{5{\cdot}000}{T} - 8{\cdot}8 \pm 0{\cdot}5 \text{ (fig. 5).}$$

(*b*) *Influence de la concentration du glucide.* Entre les concentrations 0·14 et 0·0055M le rH_2 limite des solutions de glucides est sensiblement constant.

(*c*) *Influence du milieu salin.* La concentration du tampon n'influe pas sur le potentiel final; celui-ci varie cependant avec le milieu lorsque ce milieu a une action chimique sur le glucide. C'est ainsi que, dans un tampon au borate + KCl, il y a formation de complexes: le potentiel est plus positif que dans les phosphates pour les milieux dont le *p*H est inférieur à 9, *p*H qui correspond à la valeur de la constante de dissociation de l'acide borique.

Nature du potentiel mesuré dans les solutions de glucides. Le potentiel limite s'établit très lentement en milieu neutre et à basse température; en outre, la reproductibilité des potentiels n'est pas comparable à celle que l'on obtient avec les systèmes en équilibre très rapide. Cependant les solutions de glucides, après évolution à l'abri de l'air, renferment bien un système oxydoréducteur réversible.

(*a*) *Indications colorimétriques.* Une première indication en faveur de l'existence d'un système réversible vrai est le fait que le potentiel déterminé électrométriquement permet de prévoir quels indicateurs d'oxydoréduction seront réduits par les glucides. Les colorants dont le potentiel normal d'oxydoréduction à *p*H 7 est figuré dans le tableau ci-dessous se comportent comme peut le faire prévoir la valeur du potentiel limite mesuré dans les solutions ne contenant aucun colorant (Aubel, Genevois et Wurmser (1927)).

Thionine	0·070	Colorants réduits en quelques heures à 20°
Bleu de méthylène	0·020	
Bleu de toluidine	0·020	
Vert Janus (bleu-rose)	−0·035	
Tétrasulfonate d'indigo	−0·040	
Disulfonate d'indigo	−0·120	
Bleu de Nil	−0·140	
Phénosafranine	−0·240	Colorants non réduits après 3 mois à 20°
Vert Janus (décoloration)	−0·260	
Rouge neutre	−0·340	

Tous les colorants dont le potentiel normal d'oxydoréduction est plus positif que le potentiel limite d'une solution de glucose sont réduits en quelques heures; tous les colorants plus négatifs restent sous la forme oxydée ou *même sont réoxydés s'ils sont ajoutés sous leur forme réduite.*

De même la courbe donnant en fonction du *p*H le potentiel auquel se décolore une solution de phénosafranine coupe vers *p*H 9 la courbe des glucides. Or, effectivement, la phénosafranine est décolorée dans une solution de glucose à *p*H 10 et elle ne l'est pas même après deux mois, dans une solution à *p*H 8.

(*b*) *Influence de l'addition de systèmes en équilibre rapide.* Si le système n'était pas réversible, on peut penser qu'il serait conditionné par un régime stationnaire. Le glucide céderait par exemple de l'hydrogène atomique à l'électrode. Le potentiel limite correspondrait à l'égalité entre la vitesse de cette cession et celle d'un processus de compensation qui serait, comme le suppose Dixon (1927), dans le cas de la cystéine, la réaction

$$2H \longrightarrow H_2.$$

On devrait donc s'attendre à trouver le potentiel modifié par l'addition de colorant se mettant rapidement en équilibre avec l'électrode, ce qui est contraire à l'expérience. En effet, l'introduction de faibles quantités de colorants, dans la solution, n'a aucune influence sur la valeur du potentiel final.

(*c*) *Influence de la nature de l'électrode.* Si le potentiel mesuré dans les solutions de glucides résultait d'un régime cinétique entre la vitesse de production d'hydrogène atomique et sa recombinaison, on devrait s'attendre, en utilisant des électrodes à haute surtension pour l'hydrogène, à trouver un potentiel plus négatif qu'avec le platine.

Or, le potentiel final obtenu au bout d'un temps qui peut varier de quelques mois à quelques heures est le même, quelle que soit la nature de l'électrode qui baigne dans la solution. Pt, Au, Hg, ont été essayés.

Les systèmes en équilibre dans les solutions de glucides. Tous ces faits montrent qu'il doit se former dans les solutions de glucides un système réversible, dont la nature chimique n'est pas encore connue[1], mais dont l'existence limite la progression du potentiel vers des valeurs de plus en plus négatives. Pour démontrer définitivement l'existence de corps en équilibre d'oxydoréduction dans ces solutions de glucides, il fallait obtenir, en oxydant ou en réduisant progressivement ces solutions, des variations de potentiel pouvant être représentées par une courbe en **S**. Effectivement, quand, au moyen d'un dispositif permettant d'opérer strictement à l'abri de l'air malgré la lenteur des mises en équilibre, on titre avec du ferricyanure de potassium une solution de glucose qui a évolué jusqu'à avoir atteint son potentiel limite, on obtient, en portant les potentiels en fonction des quantités d'oxydant versées, une courbe en **S** caractéristique (fig. 6), dont le palier correspond à:

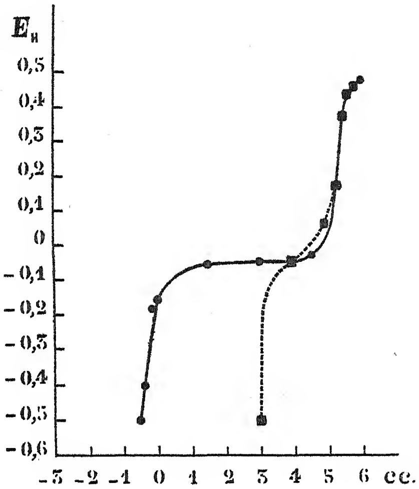


Fig. 6. Titrage d'une solution de glucide évoluée. En trait plein: titrage avec le ferricyanure (les valeurs négatives correspondent aux quantités de réducteur introduites au début de la mesure). En pointillé: titrage avec le chlorure de titane (d'après Wurmser et Geloso).

$$E_h = -0{\cdot}05 \pm 0{\cdot}02 \text{ volt à } p\text{H } 7{\cdot}2 \text{ et } 20^\circ \text{ C.}$$

soit $$rH_2 = 12{\cdot}5 \pm 0{\cdot}6.$$

Chaque point de la courbe représente un état final long à s'établir. A chaque addition d'oxydant, le potentiel s'élève brusquement, puis redescend et se stabilise au bout d'une quarantaine d'heures. Si, après avoir effectué ce premier titrage, on titre le système en retour avec un réducteur, on retrouve une courbe de même forme dont le palier est situé au même niveau mais est moins étendu.

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[1] Un système analogue se forme dans les solutions de pyruvate avec une vitesse considérablement accrue par la lumière. Il semble que, dans ce cas, il s'agisse d'une transformation énolique. Cette hypothèse a été également envisagée en ce qui concerne les solutions de glucides (Schou et Wurmser, 1928). Au point de vue proprement chimique les corps responsables du potentiel de ces solutions ont été peu étudiés. Ce sont des substances à hydrogène très mobile qui se combine à l'oxygène moléculaire, même en présence de cyanure, sauf à de hautes concentrations de cet inhibiteur (Wurmser et Geloso). En milieu acide, la combinaison avec l'oxygène est moins facile et le pyrophosphate inhibe la réaction (N. Mayer).

Cette différence entre les deux titrages est due à l'instabilité de la forme oxydée en équilibre, à une décomposition irréversible qui prolonge l'action de l'oxydant et se révèle par une décarboxylation (Wurmser et N. Mayer, 1932). Celle-ci peut être évitée par l'emploi d'un oxydant plus doux que le ferricyanure. Effectivement, en employant des solutions d'un indophénol comme oxydant on obtient, avec des corps analogues, des titrages en retour presque théoriques.

C'est ce qu'a réussi Georgescu (1932) en opérant sur l'acide hexuronique, extrait des oranges selon la technique de Szent-Gyorgyi (1928), et sur les acides glycuronique et galacturonique. Ces corps se comportent à peu près comme les solutions de glucides évoluées, mais le palier des courbes de titrage correspond à un potentiel moins négatif.

Ainsi, malgré la lenteur des mises en équilibre, la réversibilité du système formé dans les solutions de glucides n'est pas douteuse. On en peut trouver une confirmation nouvelle en ajoutant à une telle solution des colorants de potentiels égaux et inférieurs à celui du palier des courbes de titrage $E_h = -0{\cdot}05$ volt. Le bleu de méthylène dont le potentiel normal dans les mêmes conditions (pH 7·2 à 20°) est $+0{\cdot}01$ est réduit quantitativement, tandis que le bleu de Nil dont le potentiel normal est $-0{\cdot}15$ volt n'est réduit que dans la proportion compatible avec la mesure électrométrique.

Les solutions de glucides évoluées à l'abri de l'air nous offrent donc pour la première fois parmi les corps du métabolisme un exemple d'équilibre électrochimique; elles vont encore nous montrer un autre fait intéressant: quand on note les vitesses de réduction des divers colorants en présence d'une solution de glucose évoluée, on constate, comme nous l'avons vu, que tous sont réduits jusqu'à la phénosafranine non comprise, mais avec des vitesses très inégales. Il existe une discontinuité très nette au niveau du bleu de méthylène. Les colorants plus positifs, et le bleu de méthylène lui-même, sont réduits presque instantanément; ceux immédiatement plus négatifs, comme les sulfonates d'indigo, ne le sont plus qu'avec une extrême lenteur. Cette discontinuité est située dans l'échelle des potentiels à un niveau assez éloigné du potentiel d'équilibre pour qu'on ne puisse pas attribuer au voisinage de cet équilibre le ralentissement de la réduction. D'autre part, il ne semble pas que la discontinuité soit due à la nature chimique des colorants à base d'indigo puisqu'elle se reproduit lorsque l'oxydant est du ferricyanure de potassium comme le montre un titrage électrométrique rapide au moyen de ce corps (Wurmser et Geloso, 1929). Tout se passe comme s'il existait dans les solutions de glucides évoluées, en plus du système réversible en équilibre lent, un deuxième système réversible, très mobile. Le palier de la courbe de titrage électrométrique de ce deuxième système correspond à:

$$E_h = +0{\cdot}03 \text{ volt à } p\text{H } 7{\cdot}0 \text{ et } 20^\circ \text{ C.}$$

soit $$rH_2 = 15.$$

On conçoit que ce système réversible très mobile peut servir de transporteur d'hydrogène[1] tant que le potentiel du système n'est pas devenu trop positif par rapport à son potentiel normal, c'est à dire tant qu'il reste des quantités notables de sa forme réduite. S'il existe dans les cellules des systèmes de ce genre, ils peuvent donc régler certaines synthèses se faisant à des niveaux d'énergie déterminés. Nous devons donc nous demander si des systèmes analogues à ceux qui se forment dans les solutions de glucides évoluées existent dans les cellules. La réponse ne semble pas douteuse. Certains même de ceux dont il a été question, tels l'acide hexuronique, ont été extraits des cellules. Mais peut-on les mettre directement en évidence dans les milieux cellulaires?

9. *Le système réducteur des cellules.*

On constate qu'en réalité la grande masse de substance réductrice des cellules agissant sur les indicateurs est constituée par le glutathion, découvert et isolé par Hopkins (1921), et que c'est le système (protéines, glutathion) mis en évidence par Hopkins et Dixon (1922) qui domine la scène.

En effet, si l'on cherche à évaluer la quantité de substance contenue dans une cellule et capable de réduire les indicateurs de potentiel, on obtient des valeurs qui ne semblent pas pouvoir être attribuées à d'autres corps. Pour entreprendre cette évaluation, Wurmser et Rapkine (1931) ont établi un dispositif permettant d'introduire sous le microscope, dans une cellule ou un élément de cellule, des quantités déterminées de substance, c'est à dire de réaliser des microinjections quantitatives. Il suffit pour cela d'ajouter au dispositif ordinaire de Chambers, constitué par un microscope et un porte-pipette micrométrique, un éclairage latéral perpendiculaire à l'axe du microscope et une petite lentille montée, elle aussi, sur un support à mouvement micrométrique. En même temps que l'on observe dans le microscope la préparation et la projection horizontale de la pipette, on lit sur l'écran vertical les longueurs de la colonne de liquide injecté dont on peut suivre à tout instant l'écoulement. Un calibrage de la pipette à la chambre claire ou à l'aide d'un micromètre oculaire permet de calculer ensuite les volumes correspondants.

On a pu par cette méthode introduire dans le noyau d'une cellule des quantités déterminées d'une solution de 2·6-dibromophénolindophénol 0·0125 M en H_2, et en suivre la décoloration en fonction du temps. Les cellules expérimentées étaient celles de la glande salivaire du Chironome. Le volume du liquide injecté peut dépasser celui du noyau lui-même qui reprend rapidement ses dimensions normales. Le colorant reste le plus souvent localisé dans le noyau, comme on peut s'en assurer en le réoxydant par injection de ferricyanure de potassium, mais cette condition, nécessaire au dosage, n'est pas toujours réalisée.

Les premiers résultats montrent que le noyau de la cellule étudiée est environ

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[1] Ce système mobile s'oxyde rapidement et se réduit ensuite aux dépens du système lent (Wurmser et Geloso, 1929). Il est sans doute identifiable avec le "glucose actif" de Clifton et Ort (1930), avec le glucose X de Blix (1927). Schou et Wurmser (1928) avaient également appelé forme active du glucose le corps à forte absorption dans l'ultra violet qui se forme dans les solutions de glucose en même temps que le potentiel s'établit. Mais cette appellation est hasardeuse

1/40 moléculaire en H_2 mobile, et contient encore des réserves d'hydrogène rapidement mobilisable. Le tableau ci-dessous donne un exemple de dosage.

Volume du noyau	Molécule-gramme H_2	Temps de réduction
$1{\cdot}2 . 10^{-4}$ mm^3	$1{\cdot}6 . 10^{-12}$	$<$ 1 seconde
1·2 ,,	3·2 ,,	$<$ 1 ,,
1·9 ,,	5·3 ,,	$<$ 1 ,,
1·9 ,,	10·5 ,,	$<$ 1 ,,
0·8 ,,	16 ,,	5 secondes

Or la concentration moyenne des tissus en glutathion est environ $5 . 10^{-3}$ *M*, soit 10 fois moindre. On peut penser que les mesures portent sur un cas particulier, et qu'en outre une répartition homogène du glutathion dans les cellules est improbable, si bien que le glutathion seul pourrait être responsable du pouvoir réducteur observé. Mais on est conduit à une autre manière de voir en reprenant une étude analogue dans des conditions où il est possible d'effectuer des séparations parmi les constituants du milieu cellulaire. Rapkine et Wurmser, dans un travail non encore publié, ont comparé les résultats de la microinjection quantitative à ceux que l'on obtient en faisant agir des indicateurs sur un autolysat de cellules.

Quand on prépare un autolysat de cellules de levure lavées, par la méthode de Lebedeff, et que l'on sépare les cellules, on obtient un extrait très réducteur qui présente vis-à-vis des colorants le même comportement que les milieux cellulaires.

Ces milieux décolorent instantanément à l'air les indophénols. Ils décolorent aussi, mais de plus en plus lentement, et en quantité de moins en moins grande, les autres colorants jusqu'à une limite variable qui, dans le vide atteint le niveau de la phénosafranine (Aubel et R. Lévy, 1931). Il en est de même pour l'extrait de cellules de levure. Or la réduction de cet indicateur correspond très sensiblement au potentiel d'une solution de cystéine qui aurait la même concentration ($5 . 10^{-3}$ *M*) que les groupements SH libres dans l'extrait. Mais les vitesses de décoloration et les masses d'indicateur réduit sont beaucoup plus grandes que si l'on opérait avec une solution équivalente de cystéine ou de glutathion. Si, dans cet extrait, nous précipitons les protéines au moyen de sulfate d'ammoniaque à saturation, le filtrat ne possède plus que les propriétés du glutathion en solution.

Hopkins et Dixon (1922) ont montré que dans les tissus une partie de la réduction du glutathion est due aux groupements – SH fixés sur les protéines. Il n'est pas douteux que, dans le milieu cellulaire, c'est surtout à ce système (protéines, glutathion) que nous avons affaire. Et les autres systèmes ne pourraient y être décelés que par une étude cinétique très fine. Dans ces conditions, le problème important est de déterminer le potentiel caractéristique de ces composés sulfhydrilés.

La question, en ce qui concerne la cystéine, a fait l'objet de nombreux travaux. Ceux qui ont été poursuivis avec des techniques électrométriques (Dixon et Quastel (1923), Michaelis et ses collaborateurs (1929)) ont abouti à la conclusion que la cystéine seule est active vis-à-vis des métaux inertes et qu'en conséquence les potentiels mesurés dans un mélange de cystine et de cystéine ne dépendent que de la concentration de cette dernière. Tout se passe comme si la forme oxydée active était à une concentration très faible et constante, comme si les solutions de cystéine étaient toujours pratiquement saturées en cette forme active[1]. Il est possible que l'inactivité de la cystine soit limitée à des échanges vis-à-vis des électrodes inertes et qu'il n'en soit pas de même vis-à-vis d'autres accepteurs. En tout cas, au point de vue qui nous occupe et qui est l'action des groupements sulfhydrilés sur les indicateurs, on peut dire que le système constitué par les protéines et les groupements SH confère au milieu cellulaire un pouvoir réducteur analogue à celui d'un système ayant une masse importante et dont le potentiel caractéristique serait d'environ − 0·050 volt à *p*H 7.

En présence de ce système prédominant, il est malaisé de rechercher l'existence d'autres corps en équilibre d'oxydoréduction. On sait que la microinjection, quand on n'observe que l'effet immédiat de l'introduction du colorant, indique des potentiels voisins de + 0·150 volt à *p*H 7, pour des cellules largement aérées (Needham et Needham (1926), Rapkine et Wurmser (1927)), c'est à dire beaucoup plus positifs que ceux obtenus par coloration vitale (Parat, 1928) ou, ce qui revient au même, par observation prolongée du colorant injecté (Cohen, Chambers et Reznikoff, 1928; Chambers, Pollack et Cohen, 1929). Ce fait peut être dû, soit à un système oxydoréducteur en équilibre rapide, ayant un potentiel normal voisin de + 0·150 volt à *p*H 7, et existant à côté du système sulfhydrilé—on peut penser au cytochrome de Keilin (1925)—soit à la lenteur croissante de la décoloration à mesure que les indicateurs ont un potentiel normal plus rapproché du niveau déterminé par les groupements SH. Dans le premier cas, le milieu cellulaire serait assez analogue aux solutions de glucides évoluées qui renferment deux systèmes de niveaux et de mobilité différents.

10. *Les niveaux d'oxydoréduction dans les cellules.*

Nous pouvons maintenant reprendre l'hypothèse qui nous a servi de point de départ et l'examiner à la lumière des quelques données dont nous disposons. Nous venons de voir que les milieux cellulaires sont pratiquement tamponnés à un potentiel qui est de l'ordre de − 0·050 volt et correspond à $r\mathrm{H}_2$ 12. La constance de ce potentiel peut-elle rendre compte de la tendance permanente des milieux cellulaires à réaliser certaines synthèses? Le premier travail sur cette question est relatif à la formation de certains aminoacides (Wurmser, 1925). Certains auteurs avaient déjà admis l'existence d'un équilibre entre les matières protéiques constitutives des cellules et une certaine concentration en acides aminés dans le liquide intracellulaire. On pouvait aller plus loin et penser à un équilibre entre les acides aminés eux-mêmes et leurs produits d'oxydation. Déjà, *biologiquement parlant*, Knoop avait, depuis 1910, admis que l'oxydation des aminoacides qui est la première phase de destruction de ces corps dans les organismes, doit être réversible. En supposant que la synthèse d'un aminoacide se fait à partir d'ammoniaque et de l'acide cétonique correspondant par formation d'un hydrate d'iminoacide:

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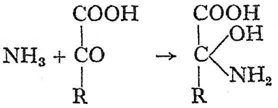
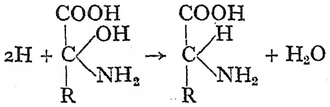
[1] Michaelis donne une autre interprétation, basée sur la formation d'un complexe entre la cystéine et le métal de l'électrode.



$$\mathrm{NH_3} + \overset{\mathrm{COOH}}{\underset{\mathrm{R}}{\mathrm{CO}}} \rightarrow \overset{\mathrm{COOH}}{\underset{\mathrm{R}}{\mathrm{C}}}\begin{matrix}\mathrm{OH}\\ \mathrm{NH_2}\end{matrix}$$

et réaction avec 2H suivant un mécanisme de Wieland:



$$2\mathrm{H} + \overset{\mathrm{COOH}}{\underset{\mathrm{R}}{\mathrm{C}}}\begin{matrix}\mathrm{OH}\\ \mathrm{NH_2}\end{matrix} \rightarrow \overset{\mathrm{COOH}}{\underset{\mathrm{R}}{\mathrm{C}}}\begin{matrix}\mathrm{H}\\ \mathrm{NH_2}\end{matrix} + \mathrm{H_2O}$$

on pouvait donc se demander si cette fixation d'hydrogène n'était pas réversible, *au sens thermodynamique*, et réglée par le potentiel de réduction du milieu cellulaire. Une évaluation grossière, la seule possible en l'absence de données sur les entropies de l'acide pyruvique et de l'alanine, montrait que l'activité de l'hydrogène en équilibre avec ses ions H^+ capable de rendre spontanée la synthèse de l'alanine, correspond à rH_2 20. D'autre part, Knoop et Oesterlin (1927) ont réussi à synthétiser certains aminoacides à partir des acides cétoniques et d'ammoniaque en présence de cystéine ou de sel ferreux. On ne réduit pratiquement avec les sels ferreux que des systèmes en équilibre avec une activité de H_2 inférieure à 10^{-18} atm. Or un résultat incontestable de toutes les mesures effectuées sur du matériel vivant est que le potentiel d'oxydoréduction des milieux cellulaires correspond toujours à une valeur de rH_2 inférieure à 20.

Le système formé par les aminoacides tels que l'alanine, et leur produit immédiat d'oxydation, est ainsi en équilibre à un niveau de potentiel assez positif pour que le milieu cellulaire tende constamment à le réduire, c'est à dire à resynthétiser l'aminoacide (Wurmser, 1925).

Pour développer utilement la théorie qui a été esquissée, il faudrait généraliser l'étude des niveaux d'énergie correspondant aux diverses synthèses, tout en s'assurant que les réactions envisagées sont bien en équilibre avec les constituants du système oxydoréducteur des cellules. Cela paraît être le cas pour la formation de l'alanine puisque la réaction peut être effectuée en utilisant la cystéine comme donateur d'hydrogène, sans catalyseur spécial.

Nous avons vu que pour une activité d'hydrogène correspondant à rH_2 6, l'acide pyruvique fixe de l'hydrogène et donne l'acide lactique. On sait d'autre part que les cellules des êtres aérobies acquièrent précisément un potentiel correspondant à rH_2 6 quand ils sont maintenus en anaérobiose (Aubel et R. Lévy, 1931). Cette limite peut s'expliquer par la concentration moyenne du glutathion dans les cellules; mais on peut objecter qu'il doit apparaître aussi dans ces cellules des donateurs d'hydrogène tels que les aldéhydes qui sont capables de réduire tous les indicateurs d'oxydoréduction jusqu'au rouge neutre inclus. On est donc tenté de voir dans la formation d'acide lactique aux dépens de l'acide pyruvique une des réactions qui déterminent ce potentiel limite. Mais ici, rien ne prouve encore que les constituants de cette réaction sont en équilibre avec le système oxydoréducteur des cellules.

Dans le même ordre d'idées, il faudrait examiner les relations trouvées entre le potentiel d'oxydoréduction des milieux de culture et le développement des microorganismes. On sait, depuis Gillespie, que dans les milieux où se développent les microorganismes anaérobies le potentiel limite peut atteindre des valeurs très négatives allant jusqu'à correspondre à une valeur de rH_2 0. Il résulte, en outre, des travaux de Quastel et Stephenson (1926), Aubel, Aubertin et Genevois (1929), Fildes (1929), Plotz et Geloso (1930) que les microbes anaérobies ne présentent une croissance rapide que si le milieu a un rH_2 compris entre 0 et 14. Cette valeur maxima est du même ordre que celle qui correspond à la grande masse du système réducteur des cellules, chez les organismes aérobies. Mais la discussion de ces résultats est très complexe; nous ne connaissons pas le potentiel intracellulaire des microorganismes en question et nous ne savons pas faire varier le potentiel de leurs milieux de culture sans y introduire en même temps des modifications qualitatives (Quastel et Wooldridge, 1929).

III. CONCLUSIONS.

Dans la première partie de cet exposé la détermination des potentiels d'oxydoréduction a été considérée comme un mode de mesure de l'énergie libre des réactions; l'importance de ces déterminations à ce point de vue n'est pas discutable.

Dans la deuxième partie, on a montré l'existence d'équilibres électrochimiques parmi les corps du métabolisme tels que les dérivés des glucides et, avec moins de certitude, parmi certains aminoacides. La teneur des cellules en substances sulfhydrilées maintient un potentiel de réduction suffisant pour rendre compte de la tendance à resynthétiser ces aminoacides aux dépens de leurs produits d'oxydation. Sans méconnaître la part d'hypothèse qui s'attache à une généralisation de ce genre, on peut penser que beaucoup d'autres synthèses participent à des équilibres électrochimiques, c'est à dire que l'hydrogène qui y intervient est en équilibre avec ses ions comme c'est le cas dans l'oxydation de l'acide succinique en fumarique, de l'acide lactique en acide pyruvique, grâce à la présence de corps électroactifs. Ces synthèses seraient alors conditionnées directement par le potentiel du milieu cellulaire. Si celui-ci reste constant, les corps synthétisés se reforment au fur et à mesure de leur consommation. On saisit ici comment sont possibles ces transformations de substances en sens inverses que les physiologistes ont décrites sans pouvoir les soumettre à aucune règle.

Ces recherches ont ouvert un chapitre nouveau de la biochimie, mais ce chapitre est à peine ébauché; il exige l'établissement de beaucoup de données expérimentales. Il sera en outre nécessaire d'examiner les relations entre ces phénomènes d'équilibre que l'on commence à découvrir et les transformations irréversibles qui caractérisent si nettement la chimie des organismes.

Les systèmes oxydoréducteurs du milieu cellulaire, en particulier le glutathion, sont eux-mêmes soumis à ces oxydations irréversibles. La constance de leur concentration dans la cellule est le résultat d'un état de régime, d'une compensation due à des actions réductrices. En apparence, ces systèmes sont peu sensibles à l'oxygène moléculaire. Par exemple en microinjectant des cellules vertes en train d'assimiler, on voit que le dégagement d'oxygène n'empêche pas les cellules de réduire instantanément les indophénols (Wurmser et Rapkine, 1927); on peut mettre en évidence le même fait grâce aux bactéries lumineuses dont la luminescence peut servir de test de la présence d'oxygène: une émulsion de bactéries lumineuses est encore brillante, c'est à dire que la pression d'oxygène est encore de $2{\cdot}6 . 10^{-8}$ atm. tandis que certains indophénols sont réduits par l'émulsion (Harvey, 1929).

Mais les systèmes oxydoréducteurs des cellules n'en subissent pas moins une oxygénation permanente.

On observe effectivement que l'anaérobiose abaisse le potentiel limite des cellules d'organismes aérobies; c'est donc que l'oxygénation compense les actions réductrices venant d'autres substances et contribue à maintenir le tampon oxydoréducteur des cellules à son niveau physiologique.

Si l'on admet que ce système oxydoréducteur règle certaines synthèses, soit directement par équilibre électrochimique, soit indirectement peut-être par action médiate sur des catalyseurs[1], on peut envisager que le maintien de ce système à un niveau d'oxydation déterminé est une part du rôle de la respiration, et l'on conçoit que, pour cette part, on puisse substituer à l'oxygène d'autres accepteurs d'hydrogène.

Ainsi certains mouvements protoplasmiques peuvent être entretenus en fournissant aux cellules un autre accepteur que l'oxygène libre. Lipschitz et Hertwig (1921) ayant immobilisé des spermatozoïdes de grenouille en les maintenant dans une atmosphère privée d'oxygène, ont vu réapparaître leur mobilité par addition de *m*-dinitrobenzène qui fixe l'hydrogène en donnant de la *m*-nitrophénylhydroxylamine.

L'oxygène libre, *bien que sa présence soit nécessaire* et que sa consommation augmente dès la fécondation, ne semble pas *entièrement irremplaçable* dans les premières phases du développement des œufs. Au début du développement de l'œuf d'oursin, le quotient respiratoire est très élevé. Tout se passe comme s'il y avait une forte décarboxylation et probablement une déshydrogénation avec fixation d'oxygène minime par rapport au dégagement de CO_2. Le rôle de l'oxygène libre est alors évidemment réduit puisque la quantité d'oxygène moléculaire nécessaire à l'œuf pour effectuer un développement normal est relativement petite par rapport au CO_2 dégagé (Rapkine, 1927).

Or, Reiss et Vellinger (1929) ont placé des œufs d'oursin fécondés dans des séries de tampons d'oxydoréduction; les œufs, au stade diaster, étaient en petite quantité par rapport aux tampons. Dans ces conditions, si l'on porte le pourcentage de développement en fonction des potentiels des tampons, on constate que les points expérimentaux se groupent selon trois zones. Le développement se fait normalement dans une première zone correspondant aux potentiels élevés. L'arrêt complet se fait dans la troisième zone pour les potentiels les plus négatifs. La région des potentiels moyens correspond à des développements partiels.

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[1] L'action différente de la cystéine et de la cystine sur certaines protéolyses diastasiques a été signalée par Grassmann, Dyckerhoff et Schoenbeck (1930). D'après Salaskin et Solowyew (1931) la cystéine accélère l'action de l'arginase et, d'après Pringsheim, Borchadt et Huffer (1931), le glutathion active la saccharification de l'amidon par l'amylase pancréatique.

D'autre part, Rapkine (1929) a obtenu un développement normal des œufs d'oursin en substituant à l'oxygène qui n'existait plus qu'à l'état de traces, de minimes quantités de bleu de méthylène.

Ces quelques faits s'interprètent bien en attribuant à la fonction respiratoire un rôle de régulateur du potentiel d'oxydoréduction cellulaire.

Il resterait à considérer un autre aspect des questions qui ont fait l'objet de cet exposé. Le milieu cellulaire est manifestement constitué par des éléments différenciés. En des territoires voisins d'une même cellule peuvent régner des équilibres à des niveaux différents. A mesure que les méthodes cytologiques permettront une exploration plus minutieuse, on devra localiser en certains de ces territoires les propriétés provisoirement attribuées à la masse totale de la cellule. Il conviendra alors de ne plus parler de potentiel d'oxydoréduction de la cellule, mais seulement du potentiel de tel ou tel de ses éléments.

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